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The present number of the JOURNAL OF EXPERIMENTAL MEDICINE, which has been edited by the undersigned, completes Volume Sixth. Articles which have accumulated during the period of suspended publication now appear, in most instances, from one to two years after their completion. Hereafter the JOURNAL will be published under the auspices of the Rockefeller Institute for Medical Research and under new editorial management. It will, as heretofore, be devoted to the publication of original investigations in the domain of the medical sciences and will, at the same time, serve as a medium of publication for the institution by which it will be issued. With the support of those who have formerly contributed and subscribed to the JOURNAL, the attempt will be made to maintain, uninterruptedly, the standard established by its first editor.

SIMON FLEXNER,
EUGENE L. OPIE.

A CONTRIBUTION TO THE PHYSIOLOGICAL DIFFERENTIATION OF PNEUMOCOCCUS AND STREPTOCOCCUS, AND TO METHODS OF STAINING CAPSULES.[1,2]

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GENERAL CONSIDERATIONS.

Pneumococci and streptococci which do not differ in morphology from their classic types can usually be differentiated from each other and identified by their morphological characters without difficulty; but it is equally true that certain cultures of these organisms, either

[1] An abstract of this paper was read at the Chicago meeting of The Society of American Bacteriologists, December 31, 1901, and published in *Ctbl. f. Bakt.*, 1902, xxxi, 302; and in *Science*, Mar. 7, 1902, 367.

[2] Received for publication March 18, 1902.

at the time of their isolation or after cultivation on artificial media, approach the type of the other so closely that it may be impossible to identify them by their morphology alone. When such morphological variations occur there are no constant and distinctive cultural or pathogenic characters as yet demonstrated, which can with certainty be depended upon as distinguishing marks between these organisms.[3]

This lack of distinct cultural differences between pneumococci and streptococci has not infrequently led to confusion, but that uncertainty should exist and mistakes be made in identification is not surprising when one considers the characters usually depended upon to distinguish pneumococci from streptococci. Chief among these, as has just been implied, are the morphological features, which are in the case of the pneumococcus a slightly lancet or elongated variation of the more typical coccus form which is characteristic of the streptococci, the arrangement of such cocci in pairs rather than in chains, and the possession of a more or less well-defined capsule. All of these characters are subject to variation or may be absent. Compared with the morphological, the cultural characters are of minor importance, and are variable. They consist in a more watery appearance of the pneumococcus colonies on coagulated blood serum and on agar, and in the usual inability of the freshly isolated pneumococcus to develop readily or at all on gelatin at temperatures below 22° C.

The distinctness of the capsule of the pneumococcus in the body fluids of man and animals, and at times when this organism is artificially cultivated in blood serum, milk or serum agar, has really been depended upon as the chief distinguishing and diagnostic character.

During the past few years, however, from time to time, instances have been reported of distinct capsule formation by organisms which had either been previously identified as streptococcus pyogenes, or

[3] On this subject see Welch, *Bulletin of the Johns Hopkins Hospital*, 1892, December, p. 125 et seq.

at the time of their isolation could not be definitely identified by their discoverers as belonging to either this group or to the pneumococci, but were considered intermediate in their character.

Brief Description of Organisms Reported as Capsulated Streptococci.—Bordet (1) working with an organism previously identified as streptococcus pyogenes described such capsule formation occurring in the peritoneal exudate of infected rabbits.

Schütz's *diplokokkus der Brustseuche der Pferde* (2), Poels and Nolen's streptococcus of contagious pneumonia of cattle (3), and especially the organism described by Bonome (4) as *"streptokokkus der Meningitis cerebro-spinalis epidemica,"* may all be looked upon as organisms differentiated on insecure grounds from either pneumococcus or streptococcus. The first two of these organisms, however, are said to be decolorized by Gram's method, and as suggested by Frosch and Kolle (5) in the case of Schütz's organism, may belong to a group intermediate between Fraenkel's diplococcus and the chicken cholera group.

Tavel and Krumbein (6) describe a streptococcus with a capsule, which was isolated from a small abscess on the finger of a child. Capsules were also present in the artificial cultures, and although ordinarily remaining uncolored, could be stained by Loeffler's flagella stain. This organism was said to be differentiated from Fraenkel's diplococcus and also in general from the streptococci (pyogenes) by a rapid and rich growth on gelatin, agar, and potato. A pellicle was formed on broth. The organisms forming this pellicle possessed capsules, but those in the deeper portions of the broth generally lacked the capsule.

In 1897, Binaghi (7) described a capsulated streptococcus isolated from a guinea-pig dead of a spontaneous peribronchitis and multiple pulmonary abscesses. In the pus were found some diplococci and short chains (4 to 6) surrounded by a capsule, which could be made evident by staining with carbol fuchsin. This organism he proposes to call Streptococcus capsulatus.

Le Roy des Barres and Weinberg (8) in 1899 published an account of a streptococcus with a capsule. This was isolated from a man who had apparently been infected from a horse which had died of an acute intestinal disorder. The patient neglected the infection and died. Diplococci and short chains furnished with a capsule were found in the subcutaneous tissue at the area of infection. The blood, liver and spleen also contained these organisms. The capsule in all the preparations remained uncolored, but the authors say that its existence was not to be doubted.

Ascitic broth inoculated from the peritoneal exudate of a rabbit dying from the infection gave streptococci in extremely long chains and surrounded by capsules. These were not so distinct as in the case of the organisms in the original smear preparations. Nothing notable was observed in the cultural characters. All fluid media (bouillon, milk and ascitic broth) were said to be strongly acid after twenty-four hours. These authors report that Achard and Marmorek have assured them that they have seen capsulated streptococci, and that Marmorek showed them some preparations in which one of his streptococci presented the same characters as that isolated by them.

Although Le Roy des Barres and Weinberg have used the term encapsulated, they believe that it would perhaps be more prudent to call their organism *streptocoque auréolé,* since they were not able to put this capsule definitely in evidence by staining it.

Howard and Perkins (9) have lately described an organism, probably of the foregoing type, which was present in a tubo-ovarian abscess and in the peritoneal exudate, the blood and some of the organs of a woman dying in the Lakeside Hospital, Cleveland, Ohio. The organisms were biscuit-shaped cocci in pairs, usually arranged in chains of four, six, eight or twenty elements, and surrounded by a wide and sharply staining capsule. In the artificial cultures special capsule stains, it was noted, failed to stain any definite area, but numerous small deeply stained granules were to be seen within the halo, especially near its outer border. Capsules in litmus-milk could be sharply stained. Howard and Perkins propose for the group composed of the streptococci of Bonome, Binaghi, and their own organism, the name Streptococcus mucosus.[4]

Reference to the original descriptions of these various capsulated streptococci will show that, with the exception of a rather poorly staining capsule, the majority of these organisms are separated from the typical streptococcus pyogenes or from the pneumococcus by exceedingly slight and unstable morphological and cultural characters. The same is true of the difference observed in their pathogenic action in animals.

There are occasions, then, both within the animal body and in artificial cultivations when it is practically impossible to distinguish definitely between some races of pneumococci and races of strepto-

[4] Through the kindness of Dr. Perkins, I have had an opportunity of studying the organism of Howard and Perkins. It ferments inulin and in most characters shows a closer affinity with pneumococci, than with true streptococci.

cocci. This difficulty is especially heightened when the pneumococcus has become non-virulent, and at the same time no very typical morphology or capsule formation is to be determined and a tendency to chain formation is marked. Cultures of pneumococci in such condition have come under my notice and were not readily to be distinguished morphologically from streptococcus cultures.

Under these circumstances there has been up to the present time no means of determining morphological or cultural differences by which these organisms could be definitely distinguished. The only recourse is the tedious attempt to revivify the pathogenicity by introduction of the culture into the most susceptible animals and to thus bring again into prominence the lancet-shaped diplococcus type, surrounded by the capsules which characterize the pneumococcus in animal fluids.

My work during the past two years with many cultures of pneumococci and streptococci from various sources has shown certain physiological differences in these organisms, which are, so far as I am able to determine, fixed characters, and have thus far proved unfailing means of distinguishing pneumococci from any streptococcus pyogenes met with. It is, of course, understood that the streptococci here referred to are organisms which would ordinarily be classed as streptococcus pyogenes (longus, brevis, conglomeratus), erysipelatos, or scarlatinæ.

In the course of these experiments I have also been led to the development of simple staining methods for capsules, and to the application of special means, cultural and otherwise, of demonstrating the presence of capsules on both pneumococci and streptococci.

It is the object of the present paper to set forth as briefly as may be these differential and capsule experiments.

THE PHYSIOLOGICAL DIFFERENTIATION OF PNEUMOCOCCUS FROM STREPTOCOCCUS.

PRELIMINARY OBSERVATIONS LEADING TO THE EXPERIMENTS IN DIFFERENTIATION.—My attention was first called to the possibility of a cultural difference between pneumococci and streptococci early in 1900. At

this time I had prepared some serum broth, composed of two parts of broth and one part of an inflammatory exudate from the human pleural cavity. This fluid had originally been rich in cells and was fairly thick, the proteid content being high. Attempts to sterilize at 68° C. had resulted in the solidification or gelatinization of the mixture, and even at 60° C. this was found to take place. The medium was finally sterilized in a fluid condition at 55° C. On March 31, I had occasion to use this medium for the cultivation of a pneumococcus which on March 24 had been isolated from some exudate coming from a case of meningitis in a child following pneumonia. In the serum-broth, after 24 hours' growth at 37° C., the organism brought about a nearly solid coagulum of a yellowish-white color. The culture was examined morphologically by the Welch capsule stain, and was found to be a pure culture of pneumococcus with clearly stained capsules. Subcultures could not be obtained from this tube after 48 hours at 37° C.

The reaction of the uninoculated serum-broth was tested and found to be 0.75% acid, phenolphthalein being the indicator. The coagulated medium containing the pneumococci reacted 2.5% acid. The coagulum dissolved upon the neutralization of the acid.

Shortly subsequent to this, tubes of this same serum-broth were inoculated with two cultures of streptococci. One of the latter was in use in immunizing experiments at the Research Laboratory of the New York Health Department, and the other was isolated by me from the exudate of an empyaema in a child. These, it was noted, did not coagulate the medium even after some days' growth at 37° C.

I was impressed with this difference in coagulative action of the pneumococcus and streptococci in this medium and, realizing that it might indicate more than a temporary difference in the metabolism of these organisms and might thus be of diagnostic value, I made further tests and endeavored to analyze the phenomenon. This, it seemed, might depend upon at least two things—either it might be due to an acid formed by the pneumococcus in the presence of some fermentable substance (probably carbohydrate) not available for the streptococci, or it might be due to an acid produced by the pneumococcus quite independent of a carbohydrate in its nutrient surroundings. That acid was formed and that the precipitate was due in large part to this was indicated by the titration and by the resolving of the coagulum upon the addition of an alkali.

What the fermentable substance was, if there really was one present, which is not unlikely, was not determined.

The normal amount of glucose in fresh beef serum, when the serum is mixed with broth in the proportion of 1 to 2 and the glucose fermented by organisms known to act upon it, such as B. coli communis and B. typhosus, does not lead to such a coagulation, neither did these organisms give a coagulum when grown in the inflammatory serum broth. It seemed, therefore, that this substance, if it were a carbohydrate, must be one of the less readily fermented, such, for instance, as glycogen; or it might be that some glucoproteid or possibly nucleoproteid, which could be broken up by the pneumococcus, was present in excess of the usual amount. This latter supposition is not unreasonable, as the cell content of the serum was large.

It did not seem probable that glycogen, even if originally present, could long remain in such a serum unchanged by the diastasic and maltasic ferments, which are normally present in blood and such exudates. Experiments were, however, carried on with various carbohydrates, with the hope of thus throwing some light on the problem, and they have led to such interesting and, it is hoped, valuable results, that they form an important part of the present communication.

No attempt has as yet been made to investigate the glucoproteids and nucleoproteids.

Culture Experiments with Serum-Broth Media, Sterilized at 65°-70° C.—In the first attempt to devise a differential culture medium, fresh beef-serum was diluted with sugar-free broth, reacting 1% acid[5] to phenolphthalein, in the proportion of two parts of broth to one part of serum. This was then divided into separate portions, one of which was left plain, and to the others were added respectively dextrose 1%, lactose 1%, saccharose 1%, dextrin 1% and starch ⅔%.

These media were sterilized at 65°-68° C. for one hour on six consecutive days. They were inoculated with two cultures of pneumococci and four of streptococci.

The dextrose, lactose, saccharose and dextrin media were found to be fermented by the streptococci as well as by the pneumococci, with the formation of a solid, yellowish-white coagulum, due to the resulting acid.

[5] This broth was neutral to litmus.

The starch medium yielded the most satisfactory differential results. This medium was rapidly fermented by the pneumococci, but was noted only after 13 days' growth at 37° C. as becoming gelatinous from the action of the streptococcus cultures. The plain serum was not coagulated by either organism. Anaërobic cultivations were made. The results were practically the same as given by aërobic growth with the exception of the coagulation of the plain serum-broth by the pneumococcus.

The results indicated by the starch medium were promising. They showed that these starch preparations could be readily fermented by pneumococci with rapid acid formation, and that the streptococci, on the other hand, although they developed in the medium, could not avail themselves of the starch, at least with ease, for it was generally a matter of many days before even a gelatinization of the medium resulted.

As there was, as determined by the iodine test and by the use of Fehling's solution, a marked conversion of the starch during the preparation of the media, and by this the introduction of complicating factors into the tests, changes were made in the mode of preparing the medium.

Culture Experiments with Serum-Water Media, Sterilized at 65°-70° C.—Distilled water was substituted for the broth in these experiments so as to exclude any hydrolization in the presence of acids and salts, such as are usually present in broth, during the preparation of the starch.

Starch water was prepared by adding 4 grams of powdered starch to 400 cc. of water, boiling for one-half hour, and allowing to stand over night. In the morning a clear fluid could be obtained by pipetting off the water from which the starch particles had settled to the bottom of the flask. This was then added to serum in the proportions of serum 1 part, starch water 2 parts, and sterilized at 68° C. for 1 hour on 6 consecutive days. A medium containing glycogen 1% was also made.

A test of these media showed that in both of them the pneumococcus not only grew readily but induced a coagulum in the glycogen as well as in the starch medium, and this often within 24 hours, at 37° C. The streptococcus cultures grew well, but, as in the first experiment, did not coagulate the starch medium readily, nor bring about coagulation of the glycogen serum, except after many days, and in some cases apparently were not able to do this at all.

Having proved by this preliminary experiment that growth of pneumococci and streptococci would take place in serum diluted with distilled water, it was possible thereafter to avoid the complicating factors incident to the use of broth.

Besides the starch and glycogen media already mentioned, tests were made also with aqueous serum media plus dextrose, lactose, galactose, maltose, saccharose. All of these mono- di- and polysaccharids were readily and rapidly used by the pneumococci, and gave rise in all cases to acid sufficient to cause the coagulation of the albuminous material in the serum. The streptococcus cultures as a rule readily fermented dextrose, galactose, and the maltose (commercial specimen), less readily lactose and saccharose; but starch and glycogen, as noted above, if changed at all, were usually only sufficiently affected by the streptococci to give rise to coagulation after many days and after some evaporation from remaining at 37° C.

These results indicated a marked difference in degree, but not necessarily in kind, between the fermentative power of the pneumococcus and streptococcus cultures as a class. No perfectly distinct differentiation, however, had so far been obtained between the pneumococcus and all of the streptococcus cultures tested.

Culture Experiments with Alkaline Serum-Water Media, Sterilized at 100° C.—It was realized that diastasic action going on during the preparation of the media might affect the result of these experiments. Changes might thus be brought about either by the diastase and maltase (glucase) and glycolylic ferments of the

blood or by the action of contaminating bacteria or their accompanying ferments during the long process of low temperature sterilization at 65°-70° C.

The diastasic action of beef serum, as was demonstrated in some experiments, in such mixtures as those used by me, was exceedingly rapid and well marked, as was also the action of maltase. Starch serum mixtures rapidly failed to give the blue reaction with iodine, and after standing for a few hours or even less at the room temperature failed to give even the brown reaction indicating the presence of erythrodextrin. Those heated to 65°-70° C. failed in these reactions even sooner. This change must be equally true in the case of glycogen, and indicated that our results were not necessarily due to the action of our organism on pure starch or glycogen, but probably on some product of the fermentation of these substances by the enzymes of the blood or by bacteria present during their preparation. Aside from these complications, low temperature sterilizations are at best tedious and unsatisfactory. An attempt was therefore made to eliminate these factors of uncertainty in the preparation of the media.

The first experiment was made by adding small amounts of alkali to the serum-water media, made as above by adding 1 part of beef-serum to 2 parts of distilled water, with the object of converting the proteids into alkali albuminates which could be boiled without coagulating.

By the addition of 1 cc. *n/1* NaOH to the serum water, a perfectly clear fluid resulted which could be sterilized at 100° C., and remain uncoagulated and clear. One-half per cent *n/1* NaOH also gave a non-coagulable medium, but was not quite as clear after boiling as the 1%. Such media are, however, open to the objection that the sugars or starches may be changed when boiled in the presence of an alkali. Gradual reductions of the alkali content were therefore practised and it was eventually found that no added alkali was needed to prevent coagulation in mixtures in the proportion of one part of ox-serum to two parts of distilled water. Such a medium,

although becoming opalescent at boiling temperature, remains perfectly fluid, and is available as a nutrient base for such sugar tests.

Before these last results were obtained, tests were made with the alkaline media plus some of the sugars, and also with the plain alkaline medium. The sugar tests showed nothing especially worth noting except that a longer interval elapsed before the coagulation of the albumins took place, this being due to the additional alkali which had to be neutralized.

The chief point of interest and value developed in connection with the use of a medium without sugar but which had been rendered slightly alkaline by the addition of 0.2% of *n/1* NaOH. In this medium the pneumococcus cultures invariably gave rise to an opalescence, a gradual whitening and final gelatinization and coagulation. The amount of alkali was afterwards reduced to 0.1% *n/1* NaOH, and this medium was found to give rise when grown in the incubator at 37° C. to the same opalescence and whitening after about 48 hours, with a firm coagulation after some days, depending upon the culture tested. As may be seen in the appended Table I, no streptococcus cultures bring about visible changes in this medium.

We thus have in this alkaline medium, made of one part of ox-serum and two parts of distilled water plus 0.1% n/1 NaOH and sterilized at 100° C., a means of differentiating the pneumococcus from various cultures of streptococci, and so far as my experience goes from all. The behavior of the pneumococcus in this medium seems to indicate a fundamental difference in its metabolism from that of the streptococci. Upon what metabolic process and nutrient ingredients this formation of acid depends has not as yet been determined.

TABLE I.

TEST OF PNEUMOCOCCI AND STREPTOCOCCI IN THE ALKALINE SERUM-WATER MEDIUM. 0.1% *n/1* NaOH.

DAYS.	PNEUMOCOCCUS CULTURES.					STREPTOCOCCUS CULTURES.
	V	VI	VII	VIII	IX	
1	..	..	..	..	..	Twenty-eight cultures were tested; none of these brought about any visible change in the medium.
2	Becoming opalescent.	Becoming opalescent.	..	Becoming opalescent.	..	
3	White.	White.	Becoming opalescent.	White.	..	
4	Gelatinous.	Gelatinous.	White.	Gelatinous.	Becoming opalescent.	
5	Semisolid.	Semisolid.	"	Semisolid.	White	
6	"	"	"	"	"	
7	"	"	"	"	"	
8	+	+	Gelatinous.	+	Gelatinous.	
9	..	..	+	..	Semisolid.	
10	..	..	..	..	+	
20	..	..	..	..	..	

+ Indicates a solid coagulum.

Culture Experiments with Serum-Water Media, Sterilized at 100° C.—After it had been observed that a mixture of one part of beef-serum and two parts of distilled water could be boiled and thus sterilized without precipitating the albuminous materials present, media were prepared in this manner, containing 1% of various carbohydrates—thus, dextrose, galactose, maltose, lactose, saccharose, dextrin, starch, glycogen, and inulin. These carbohydrates, with the exception of the maltose, which was a fair commercial sample, were of high purity, the galactose, lactose and glycogen[6] having been especially prepared and tested for this work. Such precautions, it need hardly be remarked, are absolutely essential to the success of these experiments. Samples of glycogen made by manufacturers, and bought in the open market, gave results totally at variance with those obtained when the specially prepared glycogen was tested. More readily fermentable carbohydrates were always found to be present with the glycogen in manufacturers' samples.

This series of experiments with the carbohydrates showed that the monosaccharids — dextrose, levulose, galactose — were fermented readily by practically all streptococci as well as by the pneumococci. This is true also of the disaccharids—maltose, lactose, saccharose—as regards the streptococci as a class, but the development of the coagulum may be much slower than in the case of the monosaccharids, and with certain cultures of streptococci it may be will not occur at all.[7] In the case of the polysaccharids—dextrin, starch and glycogen—coagulation usually takes place only after many days in streptococcus cultures, and in some cultures apparently does not occur. The non-coagulation is more frequent with glycogen than with starch. Inulin, however, is not fermented by any of the streptococci, although it is readily used by the pneumococci in such serum media. Growing in such an inulin medium, the pneumococcus rapidly gives rise to acid, which leads to the formation of a solid white coagulum which is

[6] These samples of glycogen, lactose and galactose were especially prepared for me by Dr. A. N. Richards, to whom my sincere thanks are due.

[7] This subject will be discussed more fully in a later paper on the fermentative power of the streptococcus group.

usually complete within 48 hours. By the use of this medium a rapid and constant differentiation can be obtained between pneumococci and the streptococcus pyogenes group.

The differences in behavior of the streptococci in inulin from their behavior in starch and glycogen—the constancy of the non-coagulation—may in part be accounted for by the character of the inulin itself. Inulin is obtained chemically pure and is therefore free from contamination with more readily fermentable carbohydrates. Furthermore, it apparently is not hydrolized during boiling in such a serum medium, nor is it affected by the diastasic ferment present in the blood serum. Glycogen, on the other hand, is only obtained in a comparatively pure state with difficulty, is probably hydrolized to some extent by boiling after it is added to the medium, and is readily acted upon by the diastase of the blood, and subsequently by the maltase, if the preparation be not rapidly carried on or the serum-water heated previous to its addition. These facts may account for some discrepant results, although some are most likely due to differences in action of the streptococci, and may indicate fundamental differences in their physiology.

Preparation of inulin serum-water medium.—The inulin medium is satisfactorily prepared as follows: To one part of fresh, clear beef-serum is added two parts of distilled water, and to this mixture is added 1% of pure inulin. The inulin goes into solution slowly in the cold, and the mixture may be warmed to 55°-60° C. to hasten this solution.[8] As soon as the inulin is dissolved, the medium should be tubed, and sterilized immediately at 100° C. for ten minutes.[9] This sterilization is repeated on the two following days. The medium prepared in this way usually becomes only slightly turbid, and no unfavorable changes occur in it.

While this is a favorable method of preparing the inulin medium and no hydrolization or change of the inulin is effected by the action

[8] The inulin may be dissolved in the distilled water before it is added to the serum.

[9] Very resistant spores are at times present in inulin. The usual three heatings are then not sufficient, and the medium should be sterilized in the autoclave, at 10 to 15 pounds for 15 minutes.

of the enzymes of the serum, it may also be prepared by first heating the serum-water mixture to 100° C. for some minutes and then adding the inulin. This method should always be followed in preparing other carbohydrate serum-water media. Litmus solution (Merck's highly purified litmus, 5% solution in distilled water) may be added to this media in the proportion of 1%, and changes in reaction thus detected by the color change.

Since sera, even from the same species of animal, differ at times in their initial degree of alkalinity and salt content, specimens are met with, though rarely, which are of so low an alkalinity or have so high a salt content that media made from them are extremely white and opaque, and near the point of heat coagulation. Such media should not be used.

The results of tests of various pneumococcus and streptococcus cultures in 1% inulin serum are shown in Table II. It will be seen that the pneumococcus cultures tested usually coagulated the serum within 48 hours, while the streptococcus cultures, 28 of which were tested, were without effect, although all of them grew at least feebly in the medium.

Summary of Culture Experiments.—It is plain from the foregoing experiments that the pneumococcus has a remarkable ability to utilize carbohydrates in its metabolic processes—all mono-, di- and polysaccharids that were tested being rapidly fermented by it as demonstrated by the production of acid. Further than this, the pneumococcus can produce sufficient acid to give rise to a coagulum in a serum medium which is sugar free, or which at least does not contain enough fermentable saccharine matter for the production of appreciable acid by such well-known fermenting organisms as the colon bacillus and the typhoid bacillus, which readily give rise to acid in the presence of glucose.

The streptococci, on the other hand, have apparently more limited and certainly less active fermentative powers. While they usually ferment the monosaccharids and in most instances the disaccharids

TABLE II.

TEST OF PNEUMOCOCCI AND STREPTOCOCCI IN THE INULIN SERUM-WATER MEDIUM.

DAYS.	PNEUMOCOCCUS CULTURES.									STREPTOCOCCUS CULTURES.
	V	VI	VII	VIII	IX	X	XI	XII	XIII	
1	+	+	+	..	..	..	+	+	..	Twenty-eight cultures were tested; none of these brought about any visible change in the medium.
2	..	..	..	+	+	..	..	..	+	
3	..	..	..	..	.	Gelatinous.	..	..	..	
4	..	..	..	..	..	"	..	..	..	
5	..	..	..	..	..	"	..	..	..	
6	..	..	..	..	..	"	..	..	..	
7	..	..	..	..	..	"	..	..	..	
8	..	..	..	..	..	+	..	..	..	
9	..	..	..	..	..	..	..	..	..	
10	..	..	..	..	..	..	..	..	..	
20	..	..	..	..	..	..	..	..	..	

+ Indicates a solid coagulum.

rapidly and with ease, the polysaccharids they either do not ferment at all or very slowly. Glycogen and starch are fermented only by certain species or races of the streptococci; inulin is fermented by none so far tested. Furthermore, an appreciable amount of acid is not produced in sugar-free serum media by any streptococcus pyogenes so far as our experience goes.

We may assume, therefore—although one hesitates to dogmatize even after extended experiments on a group of organisms whose physiology is so little understood—that there are distinct differences in respect to the physiological processes of these two forms, or to speak more correctly, of the pneumococcus on one side and the streptococcus group on the other.

The relationship of these organisms may be compared to that which exists between typhoid bacilli and the bacilli of the Gärtner-colon group. The pneumococci resemble the typhoid bacilli in the distinctness and apparent permanence of their cultural characters, while the streptococci seem to form a group which resembles the Gärtner-colon group in presenting grades of fermentative activity, and especially as this activity is displayed by the streptococci in such aqueous serum media as those used in these experiments. In making this comparison it is recognized that the pneumococcus is to be known by positive cultural characters as shown by its ability to ferment the various mono-, di- and polysaccharids, while the typhoid bacilli are chiefly distinguished in comparison with the members of the Gärtner-colon group by the absence of certain physiological activities, such as gas and indol production and the coagulation of milk. Still by this comparison it is simply desired to emphasize the co-extensiveness of the characters, as shown by these experiments, of the organisms commonly recognized as pneumococci, as compared with the variety or variability of the characters displayed by organisms which would ordinarily be classed as streptococci, and probably as streptococcus pyogenes.

EXPERIMENTS ON THE DEMONSTRATION AND STAINING OF CAPSULES ON PNEUMOCOCCI AND STREPTOCOCCI.

Welch's stain for capsules was the stain constantly used in the earlier of these experiments, and as a rule gave excellent results. Certain conditions, however, at times arose in some of the cultures which interfered with the success of this stain, and I was thus led to seek other methods of staining and of demonstrating the capsules on pneumococci.

New Methods Devised for Staining Capsules.—After many experiments it was found that a very satisfactory and rapid staining of the pneumococcus capsules could be effected by the following method:

Potassium carbonate method.—This method consists in using as a dye a half-saturated aqueous solution of gentian violet.[10] This is applied for a few seconds to the cover-glass preparation, which has previously been allowed to dry in the air, and has been fixed by heat in the usual manner. No water must be used in diluting and spreading the organisms on the cover-glass. If the organisms come from a solid medium or fluid medium other than fluid sera, some other diluting and spreading fluid such as will be described later must be used. The dye is washed off with a one-fourth per cent (0.25%) aqueous solution of potassium carbonate and the specimen is studied in this fluid. The cover-glass may be sealed on the slide by rimming with vaseline, and evaporation thus prevented. This stain gives remarkable results when used on pneumococci either from fluid or hardened serum media or from the body fluids. (See Figs. 1, 3, 4.) The capsules are large, prominent, and are either stained throughout, or their periphery appears as a dark line or layer, the part next to the deeply stained diplococci being less intensely stained than the periphery. This appearance may be due in part to a precipitation of the dye on the exterior of the capsules by the potassium carbonate.

[10] Gentian violet in substance is added in excess to distilled water and allowed to dissolve to its full extent. The solution is then filtered, and diluted to twice its volume.

Very fair capsule stains may also be obtained by simply staining for about thirty seconds with the ordinary aqueous gentian violet solution (5 cc. sat. alc. sol. to 95 cc. of distilled water) and then washing with a 1% potassium carbonate solution and studying the specimen in the fluid.

Preparations stained by the potassium carbonate method may at times give satisfactory results when dried and mounted in balsam (preferably gum Damar), but this is not the rule.

The following method, however, is eminently satisfactory when it is desired to mount specimens of pneumococci with capsules in balsam.

Copper sulphate method.—In this method a 5% or 10% aqueous solution of gentian violet or fuchsin (5 cc. sat. alc. sol. to 95 cc. distilled water) is placed on the preparation, which has been prepared as previously indicated. The dye is allowed to steam for a few seconds, by gradually passing the cover-glass downwards through the flame several times. After this the staining fluid is washed off with a 20% solution of copper sulphate. This solution may vary from 10% to saturation, but the medium strength, 20%, is generally satisfactory. After washing with copper sulphate, the preparation is dried between filter papers and when thoroughly dry mounted in balsam. The capsules remain of normal size, stained, and distinct. (Figure 2.)

Observations and Experiments on Media and Conditions Favorable to Capsule Development and Staining.—The most favorable conditions known for the development of the pneumococcus capsule are found in body fluids of man and animals suffering from an infection with this organism. For instance, capsules may be demonstrated with ease by the usual methods in the blood, serum and inflammatory exudates of the infected rabbit which is, among test animals, one of the most favorable for these experiments. Capsules may be equally well marked in the fresh sputum of pneumonia patients, especially in the early stages of the disease, and in the exudates accompanying such pneumococcus infections as meningitis, otitis media, and empyaema. In sputum and the exudates of these

various localized infections the organisms are, however, frequently degenerated or under chemical conditions unfavorable for capsule staining, and satisfactory results are then not easily to be obtained. The same is true of the scrapings from lungs of patients dead of pneumonia, often even in the stage of red hepatization. Under these conditions a longer exposure to the staining reagent is necessary, before the organisms and capsules are brought into prominence, and even then the results in nowise compare to those obtained with organisms in fresh sputum or the body fluids of such an animal as the rabbit.

It was shown by Ortman (10), as early as 1888, that outside of the animal or human body pneumococci regularly developed capsules when cultivated in blood serum. Welch [11] in 1892 and Paulsen (11) in 1893 called attention to the development of capsules on these organisms in milk, and Schmidt (12) showed that sputum media favored the formation or preservation of pneumococcus capsules. Schabad (13) made a similar observation in 1896 in regard to blood agar. Frosch and Kolle (14) refer to the demonstration of capsules on pneumococci cultivated in Guarnieri's medium, and rather indefinite statements in regard to capsules on pneumococci coming from broth and other cultivations may be found in various articles on the pneumococci.

In my own work, most of these experiments have been repeated and the results confirmed. In the serum media of various composition used during the experiments on the physiological differentiation of pneumococci and streptococci, the pneumococci not only grew readily but developed distinct and well-marked capsules. This capsule formation seems to be independent of the length of time the organism has been under artificial cultivation. One of the most favorable media of all for the development of capsules was that made of 1% starch-bouillon and serum—serum 1 part, bouillon plus 1% starch 2 parts—and sterilized at 65°-70° C. In this medium, according to my experience, capsules are developed with great regu-

[11] loc. cit.

larity and may be stained without difficulty. The same is true of pneumococci grown on Loeffler's coagulated blood serum, or on coagulated serum without glucose; and these organisms may be prepared for staining by simply spreading them on the cover-glass in some of the condensation water from the serum tube. This use of condensation water may also be successful in the case of organisms growing on plain or glycerine agar, but the most successful method to preserve or perhaps to accentuate the capsule on pneumococci or streptococci coming from artificial media, other than fluid serum media, is to use a drop of serum as the diluting and spreading fluid for the cover-glass preparations. Some of the carbohydrate serum mixtures, such as the starch or glycogen media and the alkaline media, often serve this purpose better than unmodified or fresh serum.

My first experiments in this direction were with the serum starch mixtures used for cultural purposes. It occurred to me that such a fluid in which the pneumococcocus capsules were always present and demonstrable by staining might serve to preserve or even develop them on organisms when these were transferred to a drop of the fluid from various artificial media, such as broth, agar, etc. This is successful with pneumococcus nearly without exception, and the presence of a capsule bears little relation to the time the organism has been cultivated artificially, or the medium from which it comes. It is well, however, to have fresh cultures, say usually not over 24 hours old.

It is to be noted that such a fixing medium does not bring the capsule into prominence by simply serving as a deeply stained background and leaving unstained around the organisms the so-called "retraction zone." By the potassium carbonate method the diluting medium often remains practically unstained, while the capsule stands out plainly, either stained definitely throughout, or with a distinct peripheral line or layer which shows to perfection when the organism is free from surrounding detritus. In the copper sulphate method the capsules stain uniformly while the field may or may not be free from detritus.

In smears from the serum of infected animals, *i. e.*, rabbits with pneumococcus, the potassium carbonate method leaves everything practically unstained but the organisms and their capsules. Of course, if blood cells are present they take the stain to some extent.

With the copper sulphate method the serum is stained, but is contracted into threads and masses, and does not give a uniform background. (Figure 2.)

The following experiment made with a bacillus isolated in company with a streptococcus from a case of endocarditis, but probably having no etiological significance, shows the advantage in staining capsules of preparations made with serum or serum mixtures. A smear was made directly on a cover-slip from an agar culture, no diluting fluid being used. By the potassium carbonate method a small capsule was demonstrated. When, however, it was mixed on the cover-glass with some serum (glycogen serum) and stained by the same method, very large capsules were found. In some instances the bacilli could be seen dislodged from their central position in the capsules and sticking half way out of them; in other instances the bacilli were seen entirely free and lying naked alongside of the empty capsules. The photograph of this specimen was made from a preparation stained with aqueous gentian violet, washed with 0.25% sol. of potassic carbonate, dried and mounted in balsam. (Figure 12.)

Demonstration and Staining of Capsules on Streptococci.—In determining the growth of the streptococci in the various experimental media, the cover-glass preparations were often stained by the same method (K_2CO_3) as that used to demonstrate the capsule of the pneumococcus. In this way the fact was brought to light that the streptococci of the cultures tested were possessed of capsules, which stained or were made visible by these methods. This was especially true of cultures in sugar serum media, and in the more alkaline serum media. In some cultures the capsules were quite as sharp and as well-defined as those of the pneumococcus; in others

they were less well marked, and in some appeared as if in a semi-fluid state and on the point of dissolving. In these last, when several organisms were massed together, their capsules seemed to coalesce. This, however, may occur with pneumococcus capsules, though it is by no means so frequent.

Source of the Streptococcus Cultures Used in these Experiments.—The source of the various streptococci which have been used in the experiments will be of interest in this connection, since, as was noted in the earlier part of this paper, the presence of capsules on certain streptococci has been looked upon as a character sufficient to place them in separate species.

The cultures enumerated are those which have been used in all of the cultural and capsule tests.

No. 1. Isolated from milk, May, 1898. Has been cultivated on artificial media for many months.

No. 2. Isolated from human throat (diphtheria suspected) November 18, 1898. Has been long on artificial media.

No. 3. Isolated from an abscess of a horse following subcutaneous injection of diphtheria toxin (filtered), May 17, 1900. Some months at least on artificial media.

No. 4. Isolated from another antitoxin horse. Nasal discharge due to disease of nasal sinus, probably necrosis of bone. November, 1901.[12]

No. 5. Isolated from a case of suppurative pylephlebitis in man. This organism accompanied a bacillus which was a strict anaërobe.[13]

No. 7. From a case of appendicitis.

No. 8. Culture marked "M" from the Research Laboratory, Department of Health, N. Y. Isolated from a case of erysipelas.

No. 9. Same culture as "8," but grown for many months as a separate culture under slightly different cultural conditions.

No. 10. Said to have come from a case of scarlet fever. Research Laboratory, Department of Health, N. Y. Marked "B."

No. 11. Present in pure culture in the urine of a man suffering from a marked cystitis. Patient gave history of chronic pyuria. Capsules on streptococci in the original urine smears.

[12] I am indebted to Dr. Theobald Smith for these four cultures. They were labelled I, IV, VII, X in the order enumerated in the text.

[13] See Norris, *Journal of Medical Research*, 1901, I, 97. I am also indebted to Dr. Norris for culture 7.

No. 12. Rapid ascending cellulitis of leg. Tissues after 24 hours appeared gangrenous. Patient showed marked signs of sepsis, but eventually recovered.

No. 13. Isolated from the heart blood of a patient showing lesions of endocarditis.

No. 14. Isolated from human uterus. Puerperal sepsis.[14]

These cultures from such widely different sources present the usual characters ascribed to streptococcus pyogenes, and so far as I have studied them, are not to be differentiated from one another by the ordinary cultural tests. Morphologically they do not present more than the ordinary slight differences which may be probably attributed to slight variations in physical and chemical environment, rather than to constant inherent differences in the organisms themselves.

All of them coagulate milk after a varying number of days, and grow in gelatin at the room temperature. Nos. 8, 9 and 10 developed tardily in gelatin, did not extend along the puncture to the surface of the medium, but grew along the lower half of the line of inoculation. All of the other cultures grew readily in gelatin, some of them spreading slightly away from the point of inoculation on the surface of the medium.

The broth cultures were not especially noteworthy, except in the different behavior of the same organism when tested in broth containing sugars, and in sugar-free broth. Either flocculent growth or uniform clouding may occur according to the character of the broth in which cultivations are made. Uniformly clouding non-sugar broth, they may present flocculi in one or more of the sugar broths, or vice versa, according to the culture tested. These characters seem to be independent of the acid produced in the medium. I note, for example, organism "8," *glucose broth:* uniform clouding, dense; *plain broth:* flocculi, medium amount of growth. Organism "12," *plain broth:* flocculi small, practically uniform clouding; *glucose broth:* flocculi large, plentiful sticky growth, organisms in very long chains.

All of the streptococci from the above described sources have been found to possess capsules which become apparent under various conditions when stained by the methods mentioned. Streptococcus No. 11 had a well-marked and easily stained capsule in the original urine smear preparations, and also in some of the artificial cultiva-

[14] See Wadsworth, *American Journal of Obstetrics*, 1901, XLIII, No. 4.

tions, especially that on glycerine agar, and on serum agar. The same is true of streptococcus No. 7.

The photographs of most of the preparations were taken after the organisms had been cultivated for many generations on artificial media. As a rule, the best examples were given by cultures on ascitic serum agar. My best results with streptococci were obtained with organisms coming from serum (ascitic) agar, and diluted on the cover-glass in a drop of glycogen serum mixture which had been sterilized at 68° C., and had undergone evaporation to about one-half of its original volume.[15] It happened that the chemical and physical condition of this mixture was exceedingly favorable to capsule preservation. Most of the photographs of streptococci were taken from specimens prepared in this way, dried in the air, fixed in the flame, and stained by the method in which a half-saturated aqueous solution of gentian violet and 0.25% potassium carbonate solution are used. Fine clear specimens may often be obtained in this way, when not a trace of capsule can be demonstrated on the organism in smears direct from fluid or other artificial media.

These experiments, in which it has been possible to demonstrate capsules in all of the cultures of streptococci tested and this often after long cultivation on artificial media, suggest that to place streptococci which have been found by the usual methods possessed of capsules, in a different specific group from the ordinary streptococci pyogenes is not warranted. And especially is this true if this character is the sole or major distinguishing feature.

In the present or immediately preceding history of such cultures, conditions favoring the development of capsules may have existed and thus brought the capsule formation to a maximum. Such conditions may have been found in the animal or human host, or as in the case examined by me, in such a medium as an albuminous purulent urine. Organisms coming from such sources, and cultivated on artificial media might well display this character for some time sub-

[15] No attempt has as yet been made to reproduce this medium.

sequently more highly developed than it is among their fellows which had not had the same previous history.

I have noticed that pneumococci, which it is well known have particularly well-marked capsules in rabbit blood or serum, have this character, in some instances at least, to a less degree in the serum of guinea-pigs which have succumbed to the pneumococcus infection.

It seems more advisable, therefore, in the present state of our knowledge, to look upon capsule formation as general among streptococci, and of no absolute diagnostic or differential significance. It is also fair to conclude that unless there are cultural differences accompanying this marked development of the capsule on organisms suspected of being streptococci, that such organisms should not be placed in a separate specific group.

GENERAL SUMMARY AND CONCLUSIONS.

By morphological examination and with current cultural methods a clear differentiation cannot always be made between pneumococci and streptococci. The chief differential character usually depended upon is the capsule of the pneumococcus. Well-marked capsules, however, may occur on organisms which have with reason been classified as streptococci. On the other hand, capsules may not be demonstrable on pneumococci by the usual methods, especially when growing on artificial culture media.

The usual cultural characters and reactions are at best not diagnostic, and are subject to variations which may render them useless as evidence of specific difference.

The experiments recorded in this paper, however, afford some evidence that there are well-marked differences between the metabolic activities of pneumococci and streptococci, which may prove useful in the differentiation of these organisms. These differences in metabolism become apparent when the pneumococci and streptococci are cultivated in an alkaline serum medium, or in a serum medium to which the carbohydrate, inulin, has been added.

Pneumococci slowly produce acid in the alkaline serum.

In the inulin media they ferment the inulin and thus rapidly give rise to acid. Streptococci do not form appreciable acid in either of these media, nor do they ferment the inulin.

The differences between the metabolism of pneumococci and streptococci are indicated by visible changes in the media. Thus the alkaline serum and the inulin serum are coagulated by the acid formed during the growth of the pneumococci. This coagulation is rapid in the inulin serum medium, slower in the alkaline serum medium. The streptococci, on the other hand, do not bring about a coagulation of these media.

We have, therefore, in either of these two media, the alkaline or the inulin, so far as our experience goes, a definite means of differentiating pneumococci from streptococci.

Starch and glycogen media, prepared in the same manner as the inulin medium, are coagulated by pneumococci and by some at least of the streptococci. With the streptococci the coagulation, if it occurs at all, is usually long delayed. It may be that some or all of the streptococci do not ferment pure starch and glycogen.

Lactose, saccharose and maltose are fermented by pneumococci with the production of acid, thus giving rise to acid coagula in media containing serum. Certain members, though probably not all, of the Streptococcus pyogenes group ferment these disaccharids—lactose, saccharose, maltose—hence such sugars are not available in media used to differentiate pneumococci from streptococci as a group.

Monosaccharids—dextrose, galactose, possibly all monosaccharids—are fermented by pneumococcus and the various members (probably all) of the streptococcus group. Serum media containing these sugars are rapidly coagulated by the resulting acid.

In serum media, especially starch-bouillon-serum, sterilized at 68° C., pneumococci usually develop well-marked capsules. In some of the serum media, streptococcus cultures may at times have demonstrable capsules.

All streptococci examined, which by the usual methods would be classified as streptococcus pyogenes, have been found to possess cap-

sules. These were demonstrated by special methods and stains devised during this work.

These methods and stains are also especially applicable to the demonstration and staining of pneumococcus capsules.

In the light of the demonstration of capsules on streptococci, the usual morphological basis of differentiation of streptococci from pneumococci appears insecure.

This is also true of the separation of the organisms described as capsulated streptococci into species distinct from streptococcus pyogenes, or from true pneumococci.

This separation does not seem warrantable unless other and especially well-marked cultural differences are demonstrated, which distinguish such capsulated streptococci from pneumococci or from streptococcus pyogenes. Well-marked examples of organisms which would probably be described as capsulated streptococci have been examined during these experiments. They have been found to correspond to streptococcus pyogenes when cultivated in the media described in this paper.

It is a pleasure to acknowledge my indebtedness to Dr. Edward Leaming for the photographs reproduced in the plates.

REFERENCES.

1. Bordet.—*Ann. de l'Inst. Past.,* 1897, xi, 177.
2. Schütz.—Ref. in *Centralb. f. Bakt.,* 1887, i, 393.
3. Poels u. Nolen.—*Fortschritte d. Med.,* 1886, iv, 217.
4. Bonome.—*Ziegler's Beiträge,* 1890, viii, 377.
5. Frosch u. Kolle.—Flügge's Mikroörganismen, 1896, ii, 161.
6. Tavel and Krumbein.—Ref. *Centralb. f. Bakt.,* 1895, xviii, 547.
7. Binaghi.—*Centralb. f. Bakt.,* 1897, xxii, 273.
8. Le Roy des Barres et Weinberg.—*Arch. de méd. exp. et d'anat. pathol.,* 1899, xi, 399.
9. Howard and Perkins.—*Journ. Med. Research,* 1901, iv (N. S., Vol. I, No. 1), 163.
10. Ortman.—*Arch. f. exp. Path. u. Pharmak.,* 1888, xxiv, 304.
11. Paulsen.—Ref. *Centralb. f. Bakt.,* 1893, xiv, 251.
12. Schmidt.—*Centralb. f. innere Med.,* 1893, xiv, 30.
13. Schabad.—*Centralb. f. Bakt.,* 1896, xix, 993.
14. Flügge.—Die Mikroörganismen, 1896, ii, 118.

DESCRIPTION OF PHOTOGRAPHS.

PLATE XXIV.

Fig. 1. Pneumococcus I from Loeffler's blood serum. Spread in condensation water. Stained by potassium carbonate method. 2000 ×.

Fig. 2. Pneumococcus I from heart blood of rabbit. Copper sulphate method. Mounted in balsam. 2000 ×.

PLATE XXV.

Fig. 3. Pneumococcus II from serum agar. Spread on cover-glass in serum. Pot. carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 4. Pneumococcus I from sugar free broth. Spread on cover-glass in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 5. Streptococcus I from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 6. Streptococcus II from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 7. Streptococcus IV from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 8. Streptococcus VII from glycerine agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

PLATE XXVI.

Fig. 9. Streptococcus IX from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 10. Streptococcus XI from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 11. Streptococcus XIV from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 12. Capsulated bacillus from agar. Spread in serum. Potassium carbonate method. Mounted in balsam. 1000 ×.

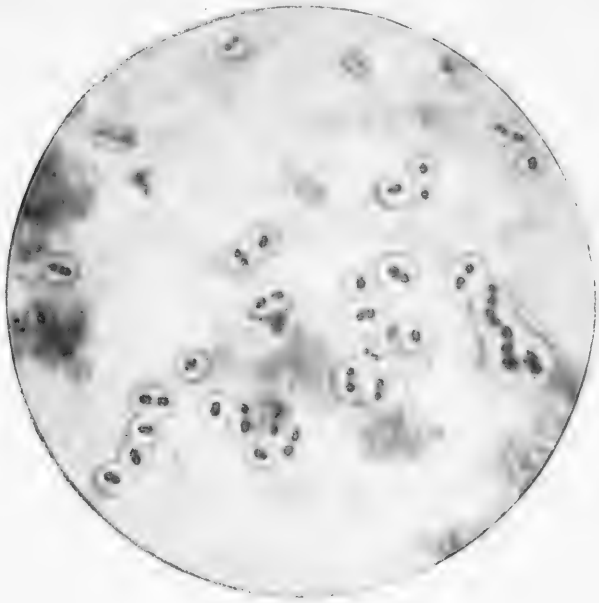

FIG. 1.

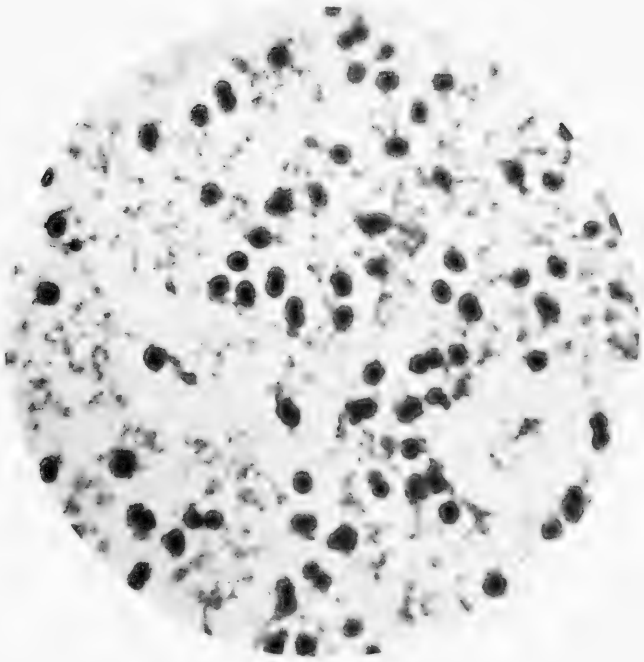

FIG. 2.

FIG. 3.

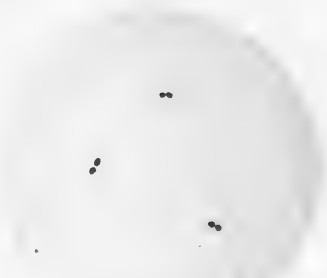

FIG. 4.

FIG. 5.

FIG. 6.

FIG. 7.

FIG. 8.

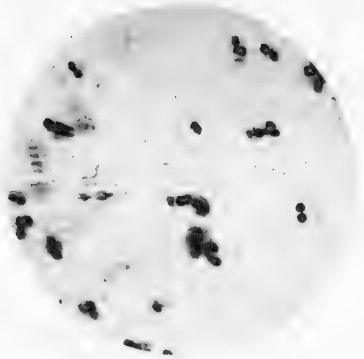

FIG. 9.

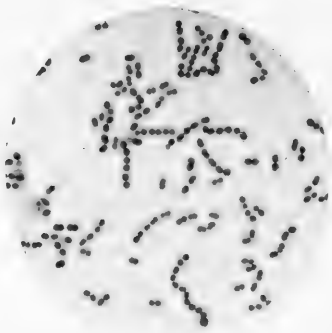

FIG. 10.

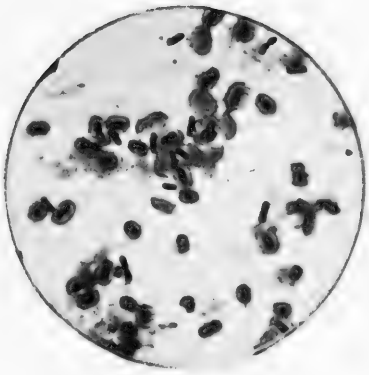

FIG. 11.

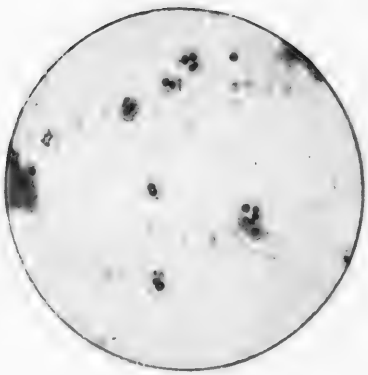

FIG. 12.

STUDIES IN HAEMOLYSIS WITH SPECIAL REFERENCE TO THE PROPERTIES OF THE BLOOD AND BODY FLUIDS OF HUMAN BEINGS.

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(*From the Royal Institute for Experimental Therapy, Frankfort-on-the-Main. Geh. R. Prof. Dr. P. Ehrlich, Director.*)

A. Comparison of the Receptors of the Red Blood-Corpuscles of Man and of the Monkey (*Makakus rhesus,* and *M. cynomolgus*).

B. Behavior of Human Blood-Corpuscles in the Presence of Serum from Various Animals, and the Haemolytic Properties of Normal Human Serum.

C. Haemolytic Properties of Human Transudates and Exudates.

A. COMPARISON OF THE RECEPTORS OF THE RED BLOOD-CORPUSCLES OF MAN AND OF THE MONKEY (*Makakus rhesus,* AND *M. cynomolgus.*)

The comparative study of the body cells of man and of those animals that are zoologically nearly related is not only of great theoretical interest, but also depends upon important practical considerations. Attention has already been called to this point by Ehrlich,[2] who said in his address at Hamburg: "I think it probable that the isotoxins will play a great rôle in diagnosis and pathology. Metschnikoff found that, when he produced a chromic acid nephritis in dogs, an isonephrotoxin developed in their blood serum, as was

[1] Aided by a grant from the Rockefeller Institute for Medical Research.

[2] Ehrlich, die Schutzstoffe des Blutes. Vortrag auf der 73. Versammlung deutscher Naturforscher und Aertzte, Hamburg, 1901. *Deutsche med. Wochenschrift,* 1901, xxvii, 865, 888, 913.

shown by the fact that this serum produced a nephritis when injected into normal dogs. It is more than probable that the most diverse isotoxins may occur in human beings—as has already been demonstrated with certainty in the case of the blood by various authors, among whom are Landsteiner and Ascoli.[3] " . . . . With the exception of experiments with the red blood-corpuscles, we can, of course, make no experiments upon the isotoxins of the body cells of man, but there are many indications that it will be possible to perform these experiments upon monkeys and thus to win a new basis for the pathology and therapy also of man."

As the isotoxins are acquiring an ever increasing interest in the study of pathological processes, it seemed profitable to investigate the relations between the receptors of man and monkey, and as the red blood-corpuscles are the cells most readily obtained, they were chosen for the study.

In this investigation the same methods were applicable that were employed by Ehrlich and Morgenroth[4] to demonstrate that there are certain receptors common to the red blood-corpuscles of goat, sheep and ox.

It was to be expected that investigation would reveal a partial correspondence between the receptors of the red blood-corpuscles of man and of the monkey, for it had already been demonstrated by means of the precipitin reaction that monkey serum and human serum contain certain specific substances in common. Thus Wassermann, Uhlenhuth, Stern, and Nuttall, all found that when rabbits were injected with human serum, their serum acquired the property of giving a precipitate when added to human serum. This precipitation was a specific reaction for human serum and occurred with the serum of no other animals except monkeys, and even in the last case the reaction was only slight. This fact shows that a part of the specific substance affected by this precipitin is common to the serum of man and monkey.

[3] See, for example, the recent work of Ascoli and Figari upon nephrolysins, *Berlin. klin. Wochenschr.*, 1902, xxxix, 560, 634.

[4] *Berliner klinische Wochenschrift*, 1900, xxxvii, 453; 1901, xxxviii, 569, 598.

Hitherto there have been no reports of investigations of other haptines of these species. In my experiments the blood and serum were obtained from two monkeys; one, *Makakus cynomolgus,* the other, *Makakus rhesus.* The human blood employed was infant's blood obtained from the maternal end of the umbilical cord. In all cases the amount employed in experiments was 1.0 cubic centimeter of a 5% suspension.

I. Action on the Blood-Corpuscles of Monkey and of Man, of Serum from an Animal Previously Treated with Injections of Human Blood.

The action of sera specific for human blood was tried upon monkey blood. It was found that the inactivated serum of a goat previously treated with human blood contains amboceptors for monkey blood and is reactivated by rabbit serum-complement better than by the complements of guinea-pig serum, human serum or monkey serum. The inactivated serum of a rabbit treated with human blood also contains amboceptors for monkey blood, and is reactivated by the complements of guinea-pig serum better than by rabbit serum, human serum or monkey serum.[5]

Interesting results were obtained from experiments to determine the smallest dissolving dose of amboceptor and of complement for human blood and monkey blood.

In the amboceptor tests, decreasing amounts of inactivated human-blood-rabbit serum were employed with a fixed amount of guinea-pig serum complement, and a fixed amount of monkey blood or human blood: 0.5 cubic centimeter guinea-pig serum was used as complement in the monkey blood experiment; 0.1 cubic centimeter guinea-pig serum as complement in the human blood experiment.

In each case 0.075 cubic centimeter of amboceptor serum was the smallest dose that effected complete solution of the blood-corpuscles.

[5] For convenience the serum of a goat previously treated with human blood corpuscles will be called "human-blood-goat serum." In the same way, specific rabbit's serum will be called "human-blood-rabbit serum," and so in other cases; the name of the material injected will be placed first in the compound word, the name of the immunised animal second.

Complement determinations were then made, using 0.1 cubic centimeter amboceptor serum for each specimen of blood with decreasing amounts of guinea-pig serum complement. The result in this case was that 0.075 cubic centimeter of complement reactivated the human blood and amboceptor, while 0.3 cubic centimeter of complement was required for the reactivation of the amboceptor with the monkey blood.

A similar result was obtained when inactivated human-blood-goat serum with rabbit serum complement was used as haemolysin, the dose of amboceptor being the same for monkey blood and human blood, the dose of complement being twice as great in the case of monkey blood as in the case of human blood.

Abundant experimental proof was obtained showing that the serum of untreated goats and rabbits possessed no such amboceptor for monkey blood as was present in the human-blood-goat and human-blood-rabbit. It was also shown that the human-blood-goat serum and human-blood-rabbit serum had no unusual action upon the blood-corpuscles from animals of other species. *In other words, it was shown that these two sera were specific for human blood, and at the same time had a specially strong action upon the blood of the monkeys employed.*

II. Action on the Blood of Monkey and of Man of Serum from an Animal Previously Treated with Injections of Monkey Blood.

Two rabbits were immunized by treatment with blood-corpuscles of *Makakus rhesus*. As only small amounts of monkey blood were available, the degree of immunity reached was not high. With each rabbit 0.025 cubic centimeter of the inactivated serum exactly sufficed to dissolve 1.0 cubic centimeter of 5 per cent suspension of monkey blood (*M. cynomolgus*), when reactivated with guinea-pig serum. These two monkey-blood-rabbit sera acted upon human blood-corpuscles only slightly more markedly than did the serum from a normal unimmunized rabbit; 0.5 cubic centimeter of the inactivated

serum producing only a "strong"[6] degree of solution of the human blood-corpuscles, when guinea-pig serum was used as complement.

Experiments showed that the haemolytic action of monkey-blood-rabbit serum upon monkey blood was destroyed by subjecting the serum to a temperature of 52° C. for one-half hour, while after one-half hour at 50° C. there was still evidence of haemolytic action.

The serum was inactivated at 52° C. and tested for amboceptor for human blood-corpuscles with a variety of sera as complement. It was found that ox serum reactivated best, that guinea-pig serum and human serum acted fairly well, rabbit serum acted very slightly, and goat serum, sheep serum and horse serum almost not at all. With ox serum as complement, 0.8 cubic centimeter of inactive monkey-blood-rabbit serum was sufficient to dissolve 1.0 cubic centimeter of human blood.

III. Relative Affinity of Human Blood and Monkey Blood for Specific Amboceptors.

An elective absorption experiment was made after the method described by Ehrlich and Morgenroth.[7]

Preliminary experiments were carried out by the method of union in order to determine the simple dissolving dose of amboceptor for human blood-corpuscles and monkey blood-corpuscles (*Makakus rhesus*). Decreasing amounts of inactivated human-blood-goat serum were employed as amboceptor; 0.3 cubic centimeter guinea-pig serum was used as complement in each case.

Amboceptor.	Human blood.	Monkey blood.
0.15 ccm.	complete	complete
0.1 "	"	almost complete
0.05 "	filmy	moderate
0.0 (complement alone)	slight	minimal

[6] The degree of haemolysis that has been effected is judged of by the color of the fluid and the amount of sediment remaining. The following terms will be used to designate the degree of haemolysis. Complete, filmy, translucent, strong, moderate, slight, trace, minimal, 0.

[7] *Berliner klin. Wochenschrift*, 1901, xxxviii, 569.

Four parallel series of tubes were then arranged with graduated amounts of inactivated human-blood-goat serum.

Two series, *A* and *B*, were treated with 1.0 cubic centimeter of 5 per cent suspension of human blood, the other two, *C* and *D*, with 1.0 cubic centimeter 5 per cent suspension of monkey blood.

After staying for one hour at 37.5° C. the corpuscles were removed by centrifugalization and the fluids tested for amboceptor for human blood and monkey blood.

Menstruums *A* and *C* were treated with the sediment of corpuscles obtained by centrifugalizing 1.0 cubic centimeter of 5 per cent suspension of human blood; menstruums *B* and *D* were treated with sediment from monkey blood. In each tube 0.3 cubic centimeter of guinea-pig serum was added as complement.

TABLE I.

Amount of human-blood-goat serum.	*A.* Solution of human blood by human-blood-goat serum, previously treated with human blood.	*B.* Solution of monkey blood by human-blood-goat serum, previously treated with human blood.	*C.* Solution of human blood by human-blood-goat serum, previously treated with monkey blood	*D.* Solution of monkey blood by human-blood-goat serum, previously treated with monkey blood
1.5 ccm.	almost complete	strong	complete	slight
1.0 "	filmy	moderate	"	"
0.5 "	strong	slight	"	trace
0.35 "	moderate	trace	almost complete	"
0.25 "	"	"	filmy	minimal
0.15 "	slight	minimal	moderate	"
0.1 "	"	"	"	"
0.05 "	"	"	slight	"
0. " (only complement.)	"	"	"	"

It is seen that human corpuscles absorb much more amboceptor than is needed to produce haemolysis, 15 times the simple fatal dose of amboceptor not being quite enough to dissolve two separate doses of human blood. It is also seen that the human red corpuscles pick up amboceptor otherwise available for monkey blood, since ten times the fatal dose of amboceptor serum after previous treatment with human blood, produced only " strong " solution of the monkey blood.

It is also evident that the monkey blood absorbs much more than the fatal dose of amboceptor, as ten times the simple fatal dose produced only "slight" solution of a second supply of monkey blood. The monkey blood absorbs more amboceptor available for monkey blood than is absorbed by human blood, for in the one case the solution of monkey blood is "slight," in the other "strong." This is merely an indication that this specimen of human blood has not receptors for all the amboceptors that have been set free in the serum of the goat as the result of a long continued course of immunization with different supplies of human blood. The monkey blood possesses receptors which the human blood specimens lack, showing affinities for certain amboceptors present in the immune serum. On the other hand, the monkey blood possesses to only a slight degree the power of removing from the immune serum amboceptor available for the human blood-corpuscles, since three and a half times the complete dose of amboceptor is almost sufficient to supply first the receptors of monkey blood, next those of human blood.

These experiments may now be summarized.

1. Serum specifically haemolytic for the human blood acts with remarkable power upon the blood-corpuscles of *Makakus rhesus* and *Makakus cynomolgus*.

2. In each case the amboceptor doses for the monkey blood and the human blood was the same, or very nearly the same.

3. Rabbit serum specifically haemolytic for monkey blood acts only feebly upon human blood. There is a great difference in its action, according to the complementing serum employed.

4. Human blood-corpuscles readily pick up from the serum specific for human blood-corpuscles amboceptors available for monkey blood, but the human corpuscles do not pick up these amboceptors as completely as the monkey blood-corpuscles themselves do.

5. Monkey blood-corpuscles pick up from the serum specific for human blood some of the specific amboceptors. This power is, however, only moderate.

IV. Action of Normal Sera upon Blood-Corpuscles of Man and Monkey.

The action of various active sera upon monkey's corpuscles (*M. rhesus*) was compared with the action of the same sera upon human corpuscles.

I. Serum from Goat.

Serum.	Monkey.	Human
1.0 ccm.	complete	complete
0.5 "	"	"
0.25 "	"	"
0.15 "	translucent	filmy
0.1 "	trace	strong
0.0 "	0	0

II. Serum from Sheep.

Serum.	Monkey.	Human.
1.0 ccm.	filmy	moderate
0.5 "	translucent	slight
0.25 "	moderate	trace
0.15 "	slight	minimal
0.1 "	trace	0
0.0 "	0	0

III. Serum from Ox.

Serum.	Monkey.	Human.
1.0 ccm.	filmy	moderate
0.5 "	minimal	slight
0.25 "	0	minimal
0.15 "	0	"
0.1 "	0	0
0.0 "	0	0

IV. Serum from Goosh.

Serum.	Monkey.	Human.
1.0 ccm.	strong	translucent
0.5 "	slight	moderate
0.25 "	0	slight
0.15 "	0	minimal
0.1 "	0	"
0.0 "	0	0

V. Serum from Rabbit.

Serum.	Monkey	Human
1.0 ccm.	0	moderate
0.5 "	0	slight
0.25 "	0	trace
0.15 "	0	minimal
0.1 "	0	"
0.0 "	0	0

Neither monkey's corpuscles nor human corpuscles were dissolved by serum from man, horse, or guinea-pig.

Summary.

1. Goat, sheep, ox, goose and rabbit serum affect monkey blood to almost the same extent that they do human blood.

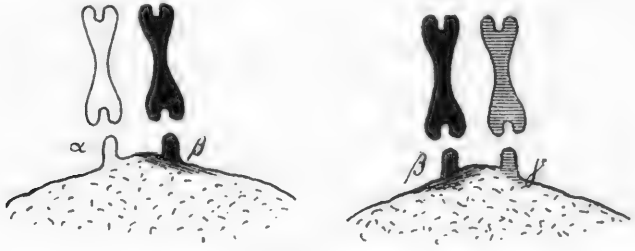
2. Human, rabbit and guinea-pig serum fail to produce haemolysis of monkey blood or human blood.

3. Monkey blood and human blood behave very similarly in the presence of normal active haemolysins.

These experiments indicate that there is quite a close relationship between the corpuscles of the human being and those of the two varieties of monkey examined. The conclusion is justified that there is a similarity of the receptor groups of the blood-corpuscles of the human being, *Makakus rhesus* and *Makakus cynomolgus*.

While there is this similarity, however, there are receptor groups in the corpuscles of the monkey which are not present in human corpuscles, and likewise the human corpuscles contain receptor groups not shared with the monkey corpuscles.

The following figure (taken from Ehrlich and Morgenroth) illustrates the relation between the receptors of human blood and monkey blood, as it is shown by the elective absorption experiment.



β represents the receptors common to the corpuscles of monkey and man.

α and γ represent the receptors peculiar to man and monkey respectively.

The partial correspondence between the receptors of the blood-corpuscles of man and monkey makes it probable that a similar rela-

tion exists between the other body cells of these animals, and, as Ehrlich has suggested, we may now be able to study the isotoxins of man by means of animal experimentation upon monkeys.

B. BEHAVIOR OF HUMAN BLOOD-CORPUSCLES IN THE PRESENCE OF SERUM FROM VARIOUS ANIMALS, AND HAEMOLYTIC PROPERTIES OF NORMAL HUMAN SERUM.

Attention is already being turned to the application of cytolytic methods in the investigation of various questions of general medical and biological importance. In view of the developments that have taken place in our ideas of immunity, it seems very important to determine the cytolytic properties of human blood according to the newer methods.

The following study of the haemolytic properties of human serum was made under the influence of this idea. Such a research should give a general idea of the cytolytic action of the serum, and furnishes a small part of the preliminary work that must form the basis of an attempt to apply the haemolytic properties of the serum to diagnosis and to questions of pathological interest.

In the recent literature there have appeared reports of several investigations along the lines here indicated.

E. Neisser and Doering[8] studied the blood from twenty persons in hospital wards, including cases of pneumonia, nephritis, tuberculosis, syphilis and emphysema. The action of the human serum upon rabbit blood was quite constant, 0.1 cubic centimeter to 0.15 cubic centimeter of serum completely dissolving 1.0 cubic centimeter of 5 per cent suspension of rabbit blood, and 0.01 cubic centimeter producing a trace of solution.

The human serum acted with decreasing strength upon suspensions of rabbit, goat, guinea-pig and pigeon blood, in the last case causing only a trace of solution. The serum from these animals showed a similar gradation in the lytic action upon human corpuscles, pigeon serum giving barely a trace of haemolysis, and 1.0 cubic centi-

[8] *Berlin. klin. Wochenschrift*, 1901, xxxviii, 593–595.

meter of rabbit serum completely dissolving the specimen of human blood.

Tests made at 0° C. and by centrifugalization showed that the haemolytic action of human serum on rabbit blood is caused by amboceptor and complement. It was possible to inactivate the human serum by heat (56° C.) and reactivate it with active rabbit or horse serum.

A specimen of human serum passed through the bacterial filter retained its complement undiminished for guinea-pig blood, but suffered loss of strength for rabbit blood, hence human serum must contain more than one kind of complement. By suitable experiments it could be shown that human serum contains separate amboceptors for rabbit and guinea-pig blood.

It was possible partly or completely to destroy the amboceptor by prolonged heat—namely, 56° C. for 45 minutes to one hour.

In contrast to E. Neisser and Doering may be cited the results published by Camus and Pagniez.[9] These investigators examined the serum from over one hundred persons and found that the action of the serum on rabbit blood varied considerably. They concluded from their results that the varying haemolytic action is an expression merely of individual differences and has no pathological value.

The preliminary report of Resinelli [10] is of interest in this connection. He investigated the differences in haemolytic power between the serum of the mother and that of the foetus. He found that the latter has distinctly less haemolytic action than the former upon the blood-corpuscles of rabbit, ox, frog and chicken.

My experiments were made with specimens of blood obtained from a maternity hospital.[11]

Upon cutting the umbilical cord, the blood flowing from the placental end was caught in a clean glass flask, defibrinated by shaking with clean iron filings, and brought to the laboratory, where the spec-

[9] *Société de Biologie*, 1901, 242.

[10] *Congresso della Società italiana di Ostetricia e Guinecologia.* Rome, 1901.

[11] I wish in this place to express my thanks to Dr. Vömel, director of the maternity hospital at Frankfort-on-the-Main, for his kindness in supplying the necessary blood.

imen was centrifugalized and the clear or blood-tinged serum was utilized as soon as possible, the sediment of blood-corpuscles being suspended in a volume of 0.85 per cent sodium chloride solution equal to the amount of serum removed, and kept on ice till needed. From this suspension, a 5 per cent suspension was made with 0.85 per cent salt solution, and 1.0 cubic centimeter of this 5 per cent suspension was the standard amount of blood used in all experiments.

The haemolytic action of various normal sera upon human blood-corpuscles was investigated. The result is recorded in Table II.

Test-tubes were arranged with a constant amount (1.0 cubic centimeter) of 5 per cent suspension of human red blood-corpuscles and decreasing amounts of haemolytic serum (from 1.0 to 0.1 cubic centimeter), and an amount of 0.85 per cent salt solution sufficient to bring the total volume of fluid up to 2.0 cubic centimeters. The several tests were made simultaneously and upon the same specimen of human blood. The test-tubes were kept in a thermostat at 37.5° C. for two hours, being shaken from time to time. They then remained in ice chests (7½° C.) over night. The results were recorded in the morning.

TABLE II.

Amount.	Goat.	Sheep.	Ox.	Horse.	Rabbit.	Guinea-pig.	Goose.	Man.
1.0 ccm.	complete	moderate	moderate	0	moderate	trace	translu-cent	0
0.5 "	"	slight	slight	0	slight	0	moderate	0
0.25 "	"	trace	minimal	0	trace	0	slight	0
0.15 "	filmy	minimal	minimal	0	minimal	0	minimal	0
0.1 "	strong	0	minimal	0	minimal	0	"	0
0.0 "	0	0	0	0	0	0	0	0

Different specimens of serum from the same species exhibit variations in haemolytic activity; for example, it happened several times that guinea-pig serum was found which exerted a distinct, though weak action upon human blood. Hence many repetitions would be necessary in order to determine the relative haemolytic strength of the sera included in Table II. It is seen, however, that goat, goose, rabbit, ox and sheep serum gave positive lytic action, while human, guinea-

pig and horse serum were negative. In other experiments it was found that eel serum is strongly haemolytic and pig serum negative.

The serum of an animal inoculated with human blood-corpuscles acquires strong lytic powers: 0.06 cubic centimeter of the inactivated specific serum of a rabbit and 0.1 cubic centimeter of the inactivated specific serum of a goat containing sufficient amboceptor to dissolve 1.0 cubic centimeter of human blood suspension; while it proved difficult to produce haemolysis of human blood with inactivated normal goat serum as amboceptor. Of the latter 0.8 cubic centimeter, using guinea-pig or rabbit complement, gave only slight or moderate solution.[12]

No special endeavor has been made to find variations in the behavior of different specimens of human blood-corpuscles. Judging from the numerous tests made with specific haemolysins and antihaemolysins, the action of the human red blood-corpuscles appears to be remarkably constant.

The haemolytic action of normal, active human serum was determined. 1.0 cubic centimeter of a 5 per cent suspension of the blood of each animal tested was treated with decreasing amounts (0.8 to 0.1 cubic centimeter) of a specimen of human serum, the quantity of fluid in the test tubes being kept constant. The experiments were made simultaneously. There was no solution of the blood-corpuscles of man, ox, goat, sheep, guinea-pig, rat or goose. Rabbit blood was completely dissolved by 0.8 cubic centimeter of serum, while 0.2 cubic centimeter showed no action.

In the course of other experiments controls were made in many cases to determine the action of other specimens of human serum upon human, ox and sheep blood. In all cases the human serum failed to show any lytic action.

In one instance that came under observation, there was a very interesting difference between the action of infant's serum and serum from the mother upon guinea-pig blood. The infant's serum in doses

[12] For the reactivation of normal inactive sera see Sachs. *Berliner klin. Wochenschrift*, 1902, xxxix, 182, 216.

up to 1.0 cubic centimeter failed to dissolve this blood, while 0.25 cubic centimeter of the mother's serum dissolved it readily. Apparently, the adult serum contains a haemolysin that is absent from the infant's serum, a condition hitherto unrecorded. It will require further investigation to determine whether this condition is due to absence of both amboceptor and complement from the infant's serum, or of only one of these constituents.

Max Neisser [13] observed a similar difference between serum from old and young horses, the former normally containing antitetanolysin and antistaphylolysin, substances which are completely or nearly completely absent from the serum of young horses, and G. Müller [14] describes the same condition in regard to the agglutinins of the serum of cattle.

From the experiments above recorded it is seen:

1. That the red blood-corpuscles of the infant are dissolved by the serum of various animals, and that the action of one kind of animal serum differs from that of another kind.

2. That infant's serum possesses but feeble haemolytic properties, acting as it does upon only one species of animal blood, namely, the rabbit's. The lytic action in the instance observed by me was weak—weaker than the action found by Neisser and Doering to be constant. This result may be due to individual differences or it may be the result of a peculiarity in infant's serum corresponding to the condition described by Resinelli. (Op. cit.)

In further experiments the complement strength of human, ox and horse serum was compared.

Preliminary tests showed that horse serum exerted no lytic action on human blood-corpuscles, while only 0.2 cubic centimeter of ox serum could be used, as 0.25 cubic centimeter brought about partial solution of the human blood-corpuscles. In one series of tests 1.0 cubic centimeter of human blood suspension and 0.8 cubic centimeter of inactivated specific goat serum were treated with the three

[13] *Deutsche med. Wochenschr.*, 1900, xxvi, 790.

[14] Müller, *Über Agglutinine Normaler Thiersera*, Inaug. Dissert., 1901.

different complements. In a second series, made simultaneously, inactivated normal goat serum, human blood and complement were brought together. After two hours in the thermostat, the specimens were kept on ice over night and the results were noted in the morning.

TABLE III.

Amboceptor.	Complement. 0.2 ccm. Ox.	0.75 ccm. Horse.	0.8 ccm. Man.
1. human-blood-goat serum: 0.8 ccm.	complete	complete	moderate
2. normal goat serum: 0.8 ccm.	moderate	0	slight
3. control: no amboceptor	trace	0	0

About three times the completely dissolving dose of inactivated human-blood-rabbit serum and of inactivated human-blood-goat serum were treated with two specimens of fresh human serum as complement.

0.8 ccm. Human serum.	Human-blood-rabbit.	Human-blood-goat.
I	complete	filmy
II	minimal	translucent

The two sera reactivated the goat serum almost equally well, but there was a great difference in the power of the first and the second to reactivate the rabbit serum.

The complement action of several sera was compared, a constant amount of complement being used with decreasing amounts of amboceptor (inactivated human-blood-rabbit serum).

TABLE IV.

Amboceptor	0.3 ccm. Rabbit.	0.2 ccm. Guinea-pig.	0.75 ccm. Horse.	0.8 ccm. Man.
0.2 ccm.	complete	complete	complete	complete
0.15 "	"	"	moderate	"
0.1 "	"	"	slight	translucent
0.06 "	filmy	"	trace	slight
0.04 "	"	filmy	0	trace
0.02 "	translucent	translucent	0	0
0.01 "	moderate	moderate	0	0
0.0 "	0	0	0	0

In this case the reactivating power of human serum was weaker than that of rabbit or guinea-pig serum and was approximately as strong as the serum of the horse.

From the experiments just recorded it is seen:

1. That human serum contains complement which will reactivate an amboceptor specifically haemolytic for human blood-corpuscles.

2. That the amount of complement varies with different specimens of serum.

3. That the complement available for one amboceptor and that available for another amboceptor may vary independently of each other.

The following further experiments were made with human serum complement:

Three fresh specimens of human serum were tested as complement for ox-blood-rabbit serum. A preliminary test with ox-blood-rabbit serum reactivated by guinea-pig serum showed that 0.0005 cubic centimeter sufficed to dissolve completely 1.0 cubic centimeter of 5 per cent suspension of ox blood. With three times this amount complement determinations were made, using decreasing amounts of the three specimens of human serum.

TABLE V.

Amount.	Serum I.	Serum II.	Serum III.
0.5 ccm.	moderate	slight	filmy
0.25 "	slight	trace	translucent
0.1 "	trace	minimal	moderate
0.05 "	minimal	"	slight
0.035 "	"	"	trace

Controls with 0.75 cubic centimeter complement without amboceptor were negative.

The complement action of the first of these specimens was also tried with other amboceptors.

Preliminary determinations, using goat serum as complement, showed that 0.075 cubic centimeter of ox-blood-goat serum, and 0.3 cubic centimeter of sheep-blood-goat serum sufficed to dissolve 1.0 cubic centimeter of ox blood and sheep blood respectively. With guinea-pig serum as complement it was found that 0.075 cubic centimeter of human-blood-goat serum was the simple lytic dose.

TABLE VI.

Amount of complement.	0.1 ccm. Ox-blood-goat serum.	0.5 ccm. Sheep-blood-goat serum.	0.3 ccm. Human-blood-goat serum.
1.0 ccm.	moderate	complete	complete
0.75 "	"	"	"
0.5 "	"	"	"
0.35 "	"	"	filmy
0.25 "	"	"	translucent
0.2 "	slight	"	slight
0.17 "	"	"	trace
0.13 "	"	"	0
0.1 "	"	filmy	0

Controls showed that 1.0 cubic centimeter of complement alone produced no haemolysis.

A similar experiment was made with another specimen of human serum, an excess of amboceptor being employed with decreasing amounts of complement.

TABLE VII.

Human serum.	0.5 ccm. Human-blood-goat serum.	0.2 ccm. Human-blood-rabbit serum.	0.012 ccm. Ox-blood-rabbit serum.	0.3 ccm. Sheep-blood-goat serum.
0.8 ccm.	0	strong	moderate	complete
0.4 "	0	slight	slight	filmy
0.2 "	0	trace	trace	translucent
0.1 "	0	0	minimal	moderate
0 "	0	0	0	0

As experiment with human serum as complement for normal haemolysis resulted as follows:

Preliminary determinations showed that 1.0 cubic centimeter of 5 per cent suspension of guinea-pig blood was completely dissolved by:

0.1 ccm. of active dog serum,
0.25 ccm. of active sheep serum,
1.0 ccm. of active rabbit serum,
0.1 ccm. of active ox serum.

The four sera were inactivated at the lowest inactivating temperature (see Sachs, loc. cit.); and used as amboceptor for guinea-pig blood.

TABLE VIII.

Complement.	0.25 ccm. Dog serum.	0.35 ccm. Sheep serum.	1.0 ccm. Rabbit serum.	0.25 ccm. Ox serum.
0.5 ccm. Human serum	complete	complete	complete	complete

The control with 0.5 cubic centimeter human serum alone had no effect upon guinea-pig blood.

All of the experiments with complement reported above were performed in the usual manner, the amboceptor, complement and blood being mixed in test tubes and kept at 37.5° C. for two hours. The following experiments indicate that this is not a reliable test of the actual complement strength of human serum.

Two amboceptor determinations were made in the usual manner, with fixed quantities of two specimens of human serum as complement, and decreasing amounts of amboceptor (inactive human-blood-goat serum). The result was as follows:

TABLE IX.

Human-blood-goat serum.	0.2 ccm. Human serum "A."	0.3 ccm. Human serum "B."
1.0 ccm.	0	0
0.75 "	0	0
0.5 "	0	complete
0.35 "	trace	"
0.3 "	complete	"
0.25 "	filmy	"
0.2 "	strong	moderate
0.0 "	0	0

In each case haemolysis was absent with large amounts of amboceptor; as the amount of amboceptor decreased the reaction reached a maximum and again decreased with insufficient amboceptor.

In order to determine the complement strength when the inhibiting action of the amboceptor fluid was eliminated, the following experiment was made on the same day and with the same materials: Amboceptor and blood-corpuscles were mixed and after one-half to one hour the fluid was removed by centrifugalizing. The corpuscles now laden with amboceptor were suspended afresh in salt solution. Complement determinations now showed that 0.035 cubic centimeter of either specimen of human serum produced complete solution. Smaller amounts of complement were not tried. 0.3 and 0.35 cubic centimeter of amboceptor were used for the first and second complements respectively.

The following day, using the same specific serum, the same blood and the second complement serum, an amboceptor determination

was made by the same method. Corpuscles and amboceptor were united, freed from serum and suspended in salt solution, as described above: 0.15 cubic centimeter of human serum was used as complement.

TABLE X.

Amboceptor.	Degree of solution.
0.14 ccm.	complete
0.12 "	filmy
0.075 "	very slight
0.05 "	0

From this table it will be seen that the presence of the serum of the immunized animal exerts an inhibiting action upon the complement, and, what is rather remarkable, seems to interfere with its own amboceptor.

The goat from which this serum was derived has been frequently injected with human blood, not completely free from serum, and the specific serum therefore contains, in addition to the specific amboceptor, an anticomplement for human serum. Whether the inhibitive action was caused by an excess of amboceptor these experiments do not show.[15]

Summary.

1. Human serum has complement available for a variety of normal and specific amboceptors.

2. The complement differs in amount in various specimens of human serum.

3. The complement strength of different specimens of human serum for different amboceptors has no definite ratio, and the complements for different amboceptors may vary independently.

4. Inactivated human-blood-goat serum inhibits the reactivation of its own specific amboceptor by human serum.

C. HAEMOLYTIC PROPERTIES OF HUMAN TRANSUDATES AND EXUDATES.

A study was made of the haemolytic properties of several ascitic and pleuritic fluids. In the recent literature but little has appeared

[15] Neisser and Wechsberg, "*Ueber die Wirkungsart bactericider Sera.*" *Münchener med. Wochenschr.*, 1901, xlviii, 697.

on this subject. Strauss and Wolff [16] studied the haemolytic action of a series of transudates and exudates, taking as a standard the power of the fluid to dissolve rabbit blood. They tried to correlate the haemolytic action and the physical-chemical condition of the fluid, and concluded that the haemolytic properties of a fluid depend in part upon the complex structure (albumen content) of the fluid. Their experiments indicated that the complexity of the fluid was only one of the factors upon which the haemolytic power depends.

Seven different fluids were examined:

Fluids I and II (ascites) were obtained from a case of cirrhosis of the liver.

Fluids III and IV (ascites) from cases in which the clinical diagnosis was unknown.

Fluid V (ascites) from a patient with myocarditis.

Fluid VI (ascites) from a patient with carcinoma of the ovaries.

Fluid VII (pleuritis) from a case diagnosed as "tuberculous pleurisy."

Haemolytic Action.

The haemolytic activity of the fluids was tested in the same manner as the human serum, 1.0 cubic centimeter of 5 per cent suspensions of blood from various animals being employed with graduated amounts of the fluid to be examined. Without detailing the experiments, the results are grouped in Table XI, the maximal and minimal degree of solution being noted. The experiments with the various fluids were made at different times.

From a consideration of Table XI it is seen that:

1. All the specimens act as haemolysins for blood from animals of several species.

2. The haemolytic strength of the various fluids differs.

3. The action of several fluids upon the several species of blood can not be expressed by a constant ratio: Fluid "A" dissolves each of two species of blood; fluid "B" dissolves the first species, but not the second.

[16] *Fortschritte der Medizin*, xx, 1, 1902.

TABLE XI.

1 ccm. of 5 per cent suspension of blood.	Fluid I.	Fluid II.	Fluid III.	Fluid VI.	Fluid VII.
Ox.	0.5 ccm. complete. 0.15 " slight.	1.0 ccm. strong. 0.5 " slight.	1.0 ccm. trace. 0.5 " minimal.	1.0 ccm. 0	1.0 ccm. slight. 0.35 " minimal.
Sheep.	0.5 " complete. 0.1 " minimal.	1.0 " moderate. 0.35 " minimal.	0.25 " complete. 0.1 " slight.	1.0 " 0	0.5 " complete. 0.1 " trace.
Guinea-pig.	0.5 " complete. 0.15 " slight.	1.0 " moderate. 0.25 " trace.	0.5 " complete. 0.1 " trace.	0.35 " complete. 0.1 " minimal.	0.35 " complete. 0.1 " slight.
Rabbit.	0.35 " complete. 0.15 " slight.	1.0 " complete. 0.5 " trace.	0.35 " complete. 0.15 " trace.	1.0 " complete. 0.1 " trace.	1.0 " filmy. 0.35 " slight.
Rat.	1.5 " trace. 0.25 " 0	1.0 " minimal. 0.5 " 0	1.0 " minimal. 0.5 " minimal. 0.35 " 0	1.0 " 0	1.0 " slight. 0.5 " 0 .....
Goat.	....	1.0 " filmy. 0.25 " trace.	0.25 " complete. 0.1 " slight.	1.0 " trace. 0.15 " minimal.	0.25 " complete. 0.1 " moderate.
Goose.	1.5 " minimal. 0.35 " 0	1.0 " minimal. 0.5 " 0	1.0 " minimal. 0.5 " minimal. 0.35 " 0	1.0 " minimal. 0.35 " minimal. 0.25 " 0	1.0 " 0 ....
Chicken.	....	1.0 " trace. 0.25 " minimal.	1.0 " strong. 0.1 " minimal.	1.0 " slight. 0.15 " minimal.	1.0 " strong. 0.1 " minimal.
Pigeon.	....	1.0 " moderate. 0.15 " minimal.	1.0 " translucent. 0.1 " minimal.	1.0 " strong. 0.1 " slight.	1.0 " translucent. 0.1 " trace.
Man.	1.5 " 0	1.0 " 0	2.0 " minimal. 1.0 " 0	1.0 " 0	1.0 " 0 .....
Horse.	....	1.0 " filmy. 0.25 " minimal.	1.0 " filmy. 0.1 " trace.	1.0 " complete. 0.1 " trace.	0.25 " complete. 0.1 " slight.
Pig.	....	1.0 " minimal. 0.5 " 0	1.0 " minimal. 0.5 " 0	1.0 " 0	1.0 " 0 .....

4. In general, the action of the various fluids, except fluid VI, which acts exceptionally with ox and sheep blood, is very similar.

5. Haemolysis was absent, or of minimal degree, with the blood of man, pig, rat and goose.

6. The lytic action of the fluids is much stronger than the lytic action of the infant human serum examined.

Complete records of the agglutinating action of the fluids were not made. Agglutination was very frequently observed, however. For example, fluid VI agglutinated the blood of the ox, sheep, rabbit, guinea-pig, rat and goose and did not agglutinate the blood of man, chicken, pigeon or goat.

The several fluids exhibited unequal agglutinating powers.

Complement Content.

Complement tests were conducted with the fluids in the manner described when considering human serum, and the results of the tests are grouped in Table XII.

Constant amounts of amboceptor and blood suspension were employed with graduated amounts of the fluid used as complement.

In all cases where the body fluids were tried upon human blood infant's blood was employed.

Experiments made with fluid III gave results like those with human serum complement.

When amboceptor, 0.5 cubic centimeter inactive human-blood-goat serum, suspensions of human blood and complement (ascitic fluid III) were mixed, the complement determination gave the following result:

Complement (ascitic fluid III).	Result.
0.5 ccm.	complete solution
0.35 "	trace
0.3 "	minimal
0.25 "	0

The following day, with the same materials, an amboceptor determination was made. Amboceptor and corpuscles were mixed and after one-half hour the laden corpuscles were recovered by centri-

TABLE XII.

Complement.	Human-blood-goat serum amboceptor with human blood corpuscles.	Ox-blood-goat serum amboceptor with ox blood corpuscles.	Sheep-blood-goat serum amboceptor with sheep blood corpuscles.	Ox-blood-rabbit serum amboceptor with ox blood corpuscles.	Horse-blood-goat serum with horse blood corpuscles.
Fluid I	0.35 ccm. complete. 0.1 " trace. Fluid I alone, 0.	....	No reactivation.	No reactivation.	....
Fluid II	....	1.0 ccm. translucent. 0.1 " slight. Fluid II alone, 0.	No reactivation.	No reactivation.	....
Fluid III	0.1 ccm. complete. 0.02 " slight. Fluid III alone, 0.	No reactivation.	....	0.1 ccm. complete. 0.012 " 0. 1.0 " (alone) 0	....
Fluid VI	0.5 ccm. moderate. 0.35 " 0. Fluid VI alone, 0.	0.2 ccm. moderate. 0.5 " 0. Fluid VI alone, 0.	0.5 ccm. complete. 0.05 " moderate Fluid VI alone, 0.	0.15 " complete. 0.035 " slight. Fluid VI alone, 0.	No reactivation.
Fluid VII	0.5 ccm. filmy. 0.25 " 0. Fluid VII alone, 0.	0.5 ccm. filmy. 0.75 " 0. Fluid VII alone, 0.	0.35 ccm. complete. 0.035 " moderate. 0.5 " fluid VII alone, 0.	0.25 ccm. complete. 0.035 " trace. Fluid VII alone, 0.	No reactivation.

It was found in addition that Fluid II reactivated sheep-blood-rabbit serum and rabbit-blood-goat serum, and did not reactivate inactivated normal dog, sheep or ox serum for rabbit blood. Fluid VI did not reactivate goat or ox serum for rabbit blood, and reactivated both sera very slightly for guinea-pig blood.

fugalization, suspended afresh in salt solution and treated with complement, consisting of 0.5 cubic centimeter of ascitic fluid III.

AMBOCEPTOR.

Human-blood-goat serum.	Degree of solution.
0.1 ccm.	complete
0.075 "	filmy
0.02 "	minimal
control (complement alone)	0

Finding that 0.1 cubic centimeter was the simple completely lytic dose of amboceptor a complement determination was made in the same manner.

Human corpuscles were treated with 0.1 cubic centimeter of amboceptor, the excess of fluid was removed by the centrifugalization, and the laden corpuscles, freshly suspended, were treated with graduated amounts of complement.

Complement (ascitic fluid III).	Degree of solution.
0.1 ccm.	complete
0.075 "	filmy
0.03-0.02 "	slight
0.0 "	0

It is seen here that the presence of the specific serum throughout the reaction makes it necessary to employ five times as much amboceptor and five times as much complement as would be needed were the inhibitive action of the specific serum eliminated.

Experiments described in another article [11] proved that the ascitic fluids exert but very feeble antiamboceptor action upon the human-blood-goat serum, and, therefore, all or very nearly all of the inhibitive action depends upon the amboceptor serum, as in the case considered when dealing with complement of human serum.

Summary.

1. The body-fluids examined contain complements capable of reactivating various amboceptors.

2. The reactivating power is not equal in the several specimens.

[11] Marshall and Morgenroth, *Centralblatt f. Bakteriologie und Parasitenkunde, erste Abtheilung*, 1902, xxxi, 570.

3. The complement strength for a given lysin may vary in several fluids independently of the complement strength for other lysins.

4. In order to determine the actual complement strength of the fluid any inhibiting action of the amboceptor fluid must be eliminated. The same precaution is necessary in order to determine the simple completely lytic dose of amboceptor.

5. The complement action of the body fluids seems more pronounced than that of the human serum examined.

Amboceptor Content.

The several fluids were inactivated by heating to 56° C. for half an hour and examined for amboceptor. No complement action remained after this procedure.

Fluid I was devoid of amboceptor for human red corpuscles.

When ascitic fluid and complement (goat serum or guinea-pig serum) and blood were mixed no amboceptor appeared for ox blood or sheep blood. By the method of union, however, amboceptor was found. Corpuscles and ascitic fluid I were mixed, and after one-half hour the corpuscles were recovered by centrifugalization, washed with salt solution, again centrifugalized, suspended afresh in salt solution and treated with 0.15 cubic centimeter guinea-pig complement.

	Degree of Solution.	
Amboceptor (ascitic fluid I).	Ox blood.	Sheep's blood.
1.0 ccm.	complete	complete
0.5 "	"	moderate
0.25 "	"	slight
0.1 "	filmy	trace
0.0 "	0	"
(complement alone)		

On the same day and with the same materials the following experiment was performed:

Amboceptor for ox blood was removed by treating ascitic fluid I with ox blood-corpuscles freed from serum by several washings with salt solution.

Ascitic fluid thus prepared was recovered by centrifugalization and tested for amboceptor for sheep blood, controls showing that this fluid no longer contained amboceptor for ox blood.

As complement 0.15 cubic centimeter of guinea-pig serum was employed.

Ascitic fluid.	Degree of solution of sheep's blood.
1.0 ccm.	moderate
0.5 "	slight
0.25 "	trace
0.1 "	"
0.0 "	"
(complement alone).	

Summary.

1. Ascitic fluid I contains no amboceptor available for human blood-corpuscles.

2. Ascitic fluid I contains amboceptor for sheep's blood and ox blood.

3. The inhibitive action of ascitic fluid I conceals the presence of the amboceptor when guinea-pig serum or goat serum is used as complement.[18]

4. There are at least two groups of amboceptors in ascitic fluid I. One with affinity for ox blood and one for sheep blood. (In removing the amboceptor for ox blood the ascitic fluid is somewhat diluted with salt solution. The weakened amboceptor action seen in the second sheep blood experiment may be due to this dilution alone; or it may be due in part to a third amboceptor group with affinities for both ox blood and sheep blood.)

An experiment made with ascitic fluid III may be recorded at this point.

Tests were made to learn at what temperature the haemolytic action of fresh active ascitic fluid III occurs; and to determine whether there is union of corpuscle and haemolysin at temperatures too low for haemolysis to occur.

Tubes containing ascitic fluid III and rabbit blood were kept at various temperatures, and after two hours the degree of solution was noted. The corpuscles and fluid were separated by centrifugalization, the corpuscles suspended afresh in salt solution and to the

[18] Marshall and Morgenroth, loc. cit.

menstruum was added the sediment obtained by centrifugalizing 1.0 cubic centimeter of rabbit blood suspension. The tubes were then kept for two hours in the thermostat, and remained on ice over night. The results were recorded in the morning.

Preliminary determinations showed that 0.35 cubic centimeter exactly dissolves 1.0 cubic centimeter of 5 per cent suspension of rabbit blood. 2.0 cubic centimeters of ascitic fluid III and 1.0 cubic centimeter rabbit blood were employed.

TABLE XIII.

Temperature.	After 2 hours.	Menstruum.	Sediment.
5° C.	0	complete	0
10° C.	0	"	0
15° C.	trace	"	0
20° to 38° C.	complete	....	....

This experiment shows that:

1. With ascitic fluid III haemolysis of rabbit blood occurs between the temperature 15° and 20° C.

2. Both amboceptor and complement of ascitic fluid III require a minimal temperature of from 15° to 20° C. in order for union to occur with rabbit blood.

3. Amboceptor and complement of ascitic fluid VII can not be separated by this procedure.

For the sake of brevity the experiments with the various fluids upon blood of man, ox and sheep are tabulated.

The experiments were made by the method of union, using guinea-pig serum as complement. The amount of ascitic fluid used is noted in the table.

TABLE XIV.

Fluid.	Degree of solution of human blood.	Degree of solution of ox blood.	Degree of solution of sheep's blood.
I	1.0 ccm. = 0	0.25 ccm. complete	1.0 ccm. complete
II	1.0 " = 0	1.0 " slight	1.0 " translucent
III	.....	1.0 " trace	0.1 " complete
IV	2.0 " = 0	.....	1.0 " moderate
VI	2.0 " = 0	2.0 " complete	1.0 " complete
VII	2.0 " = 0	0.5 " "	0.25 " "
			lowest complete dose not reached

Controls made without removing the ascitic fluids by centrifugalization gave, for the most part, no solution, as was described for fluid I; but with fluids II, III, V and VII an amboceptor for sheep blood appeared when the fluid, sheep's blood and guinea-pig complement were simply mixed and allowed to stand in the thermostat. The same result occurred with fluid VII and ox blood. In all of these cases much stronger amboceptor action was obtained by the method of union.

Other amboceptor experiments will be given briefly:

Fluid II failed to show amboceptor for human blood when reactivated by fresh ox, goat, sheep or rabbit serum. With fluid II and ox blood the rabbit complement acted slightly. The other three sera gave negative results.

Fluid III showed no amboceptor for ox or rabbit blood when ox, goat, or rabbit serum were used as complement.

Fluid V showed no amboceptor for human or ox blood with human serum as complement.

Fluid VI showed no amboceptor for chicken or goose blood with guinea-pig, ox, goat or rabbit serum as complement.

Fluids VI and VII were tried as amboceptor for several bloods, with guinea-pig serum as complement.

TABLE XV.

Blood.	Fluid VI.		Fluid VII.	
Rabbit	1.0 ccm.	minimal	1.0 ccm.	filmy
Goat	0.25 "	complete	0.25 "	complete
Horse	2.0 "	0	2.0 "	translucent
Guinea-pig	2.0 "	0	2.0 "	slight

Smaller doses of fluid were not employed.

In all cases examined (fluids II, III, IV, VI) it was found that amboceptor for sheep blood was not removed when amboceptor available for ox blood was removed.

From these experiments it is seen that:

1. The pathologic body fluids contain amboceptor for a variety of bloods.

2. The amboceptor content of several fluids for a given variety of blood may vary independently of the amboceptor content for another variety.

3. There is more than one group of amboceptors in the fluids.

4. Different fresh sera act in different degrees as complements for the fluids.

5. No amboceptor was found available for human blood.

EPITHELIOMA ADAMANTINUM.

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An effort is made to include here the more important literature of the subject of this article appearing since 1884. Many cases are reported under titles that make them difficult to find. Others are not described with sufficient accuracy for their certain recognition. The description of the paradental epithelial débris (débris épithéliaux paradentaires) and the pointing out of its probable relation to these tumors by Malassez (1) in 1885 gave to their pathology a more logical and firmer basis than it had previously possessed. Little has been added to knowledge of the subject since that time. The most notable contribution is perhaps that of Chîbret (2), who described the formation of enamel and of cemento-dental tissue in one of these tumors. In 1897 there was published by Goebel (3) a review of the literature on all tumors of the jaw bones referable to the dental system. The majority of the contributions appearing before 1885 are, to a considerable extent, only of historical value. The statistics here presented are based upon twenty-two cases. A brief synopsis of these cases is appended. I have had the opportunity of studying the following case.

Abstract from the Clinical History.—The patient, female, aged thirty years, was admitted to St. Joseph's Hospital. Two years and three months ago she first noticed a small, hard tumor near the first bicuspid tooth on the left side of the lower jaw bone. It grew slowly. In childhood there was nothing unusual about her teeth, which were perfectly regular and even up to the time of her present illness. Examination shows that the tumor extends from angle of jaw nearly to the median line.

The dentist of the patient furnished the following history. Two years and three months ago the patient consulted him for what she considered to be an abscess in connection with a tooth. The enlargement

was apparent both inside and outside the mouth and was quite painful. As nearly as he remembers the teeth were all intact, the crowns being normal and no fillings having been introduced. The inferior maxillary bone was enlarged in the neighborhood of the first and second molar teeth, both of which were loose. On extracting the second molar no fluid appeared. On extracting the first molar there was a considerable discharge of thin, brown fluid without odor (evidently the contents of a cyst). The roots of the extracted teeth were almost absent. The patient was referred for treatment to St. Joseph's Hospital, where an operation was performed by Dr. Nathan Jacobson. Six months after operation there is no evidence of recurrence.

Abstract from the Pathological Record.—The specimen consists of part of the left lower jaw bone, extending from the angle nearly to the median line. The enlargement begins about 3 cm. anterior to the angle of the jaw and involves the bone for a distance of 7 cm. The tumor measures 7 x 3 cm. It is covered with a thin shell of bone.

There are at my disposal for description a few small blocks of tissue from the tumor fixed in Zenker's fluid and decalcified in five per cent nitric acid. In this tissue the cystic quality of the tumor is evident. The largest cyst seen is 7 mm. in diameter and is empty. Sections are cut in paraffin 6 μ thick, and stained with eosin and methylene blue.

Microscopical Examination.—Many of the sections show the mucous membrane of the mouth and a thin shell of bone and fibrous tissue surrounding the tumor. The layer of bone is interrupted in many places. The areas of bone are partially surrounded by osteoblasts and an occasional osteoclast is seen. The tumor itself consists of a connective-tissue stroma, in which there are alveoli formed by epithelial cells. The appearance under low power is well represented in Borst's (4) plates. The epithelial elements represent the enamel organ and are largely in the stage corresponding to the greatest development of the stratum mucosum (Fig. 1). One or two areas, somewhat removed from the periphery, represent the stage immediately preceding the development of the stratum intermedium and the stratum mucosum (Fig. 2, c). No definite karyokinetic figures are seen here, but some nuclei stain more deeply than others and have a

slightly ragged surface. This is represented at d, Fig. 2, where, apparently, the stratum intermedium is beginning to develop. Intercellular bridges, perhaps corresponding to those of the epithelium of the mucous membrane of the mouth, are seen. The appearance here suggests carcinoma. In many places the stratum mucosum is largely replaced by cysts containing a finely granular material staining with eosin. Anastomosis of the alveoli suggests that the epithelial constituents form a solid framework similar to that which has been shown to exist in carcinoma by means of reconstructed serial sections.

In the large alveoli is seen an external layer of cells, which in some places are cylindrical, in other places cubical (Fig. 1, a). The layer is occasionally invaginated and therefore appears in the section to be situated inside of the alveolus. The cylindrical cells perhaps correspond to the inner epithelial layer of the enamel organ, the cubical cells to the outer epithelial layer. Often, but not regularly, within the external layer are one or more layers of flattened cells, which tend little by little to assume the stellate form and correspond to the stratum intermedium (Fig. 1, b). Occupying most of the interior of the solid alveoli is the most characteristic feature of the tumor, the stratum mucosum, or enamel pulp, consisting of anastomosing stellate cells (Fig. 1, c). When seen under a lower power it might be mistaken for mucoid tissue and, especially when present in large areas, might lead to a diagnosis of myxoma. There are invaginations of the external layer of cells which with the adjacent stroma simulate the "Anlagen" of teeth in their early stages (Fig. 1, a). This gives to the alveoli the appearance of gland tubules in a stroma of mucoid tissue (Fig. 1), especially in places where the invaginated stroma has largely lost its fibrillar character and appears homogeneous (Fig. 1, d). Evidences of karyokinesis are seen in the external layer, and to a less extent in the stratum intermedium.

Various stages in the development of cysts are well seen. They are due evidently to a hyaline and granular degeneration of the stellate cells and to an accumulation of fluid between these cells. The formation of the stratum mucosum is apparently associated with

an accumulation of fluid between the cells, the formation of long processes of the cellular protoplasm, and the gradual disappearance of the intercellular bridges (Fig. 1). No evidence of enamel, dentine, or cement is seen. Chîbret (2) has described the formation both of enamel and of cemento-dental tissue in a similar tumor. The stroma (Fig. 2, a) consists of dense connective tissue in which only a few blood vessels (Fig. 2, bb) are apparent.

The tumors under consideration probably originate from structures described by Malassez (1) as paradental epithelial débris (débris épithéliaux paradentaires). A rational theory of the histogenesis of this class of tumors dates apparently from the publication of his first article. He carefully describes and illustrates these cell masses as they occur in the adult and discusses their histogenesis. He attempts to explain how epithelial tumors may originate in the bodies of the jaw bones at a considerable distance from surface epithelium. In the intra-alveolar tissue surrounding the roots of normal teeth he has found masses of cells, and from a study of the developing jaw he concludes that these masses represent the remains of the dental ridge and some of the epithelial structures originating from it, especially the neck and the outer epithelial layer of the enamel organ. A consideration of the histogenesis of adamantine epithelium suggests the possibility that adamantine tumors may arise from the gingival epithelium and from any of the derivatives of the dental ridge. The cellular masses described by Malassez are distributed in the alveolo-dental ligament from the apices of the roots to the epithelium covering the gums. The "débris" may be present in the marrow spaces of the jaw bones entirely outside the alveolo-dental ligament. This fact furnishes an anatomical basis for the origin of tumors of the jaw bones more or less independent of teeth.

Efforts have been made to associate the etiology with irregularities in the development of the teeth, with inflammatory processes, trauma, etc.; but these conditions are probably secondary rather than primary.

The sex of the individuals affected is stated in eighteen recorded cases, eight occurring in men and ten in women. The age when

the tumor was first noticed could be estimated in sixteen cases. The youngest individual was eight years of age and the oldest, fifty-eight. The greatest number of patients, five, were in the fourth decade. In two other cases the specimens are described as consisting of the jaw bones of adults. It has been thought that these tumors are prone to occur in young individuals, especially during the period of dental development. The description and figure from a case described by Massin (8) as congenital do not give evidence that he observed a tumor of this class.

The location of the tumor is mentioned in twenty cases; seventeen times it was situated in the lower jaw, twice in the upper. Becker (5) has called attention to a possible influence of the difference in anatomical relations. In the maxilla perhaps such a tumor might grow into the sinus and not cause any apparent swelling or other prominent disturbances; possibly indefinite neuralgic and other symptoms might be caused in this way.

The situation appears to be as frequent on one side as on the other. In the lower jaw the main mass of the tumor is most frequently at the angle of the jaw. From here it may extend upward to the articular surface and into the coronoid process and ventrally as far as the median line. In one case the tumor is said to have implicated one entire inferior maxilla; twice the location was in the body ventral to the angle and four times its position was median. Of the two cases affecting the upper jaw, both were on the left side and in one of these the sinus had been invaded. In Case No. 13 (see Table) both sinuses were invaded and both sides of the lower jaw bone were implicated. A median position in the upper jaw is not mentioned.

These tumors develop in the interior of the jaw bone which remains as a thin parchment-like covering. At the time of operation they have varied in size from that of a plum to that of the head of a foetus, but are most frequently about the size of a hen's egg. In two cases it is stated that the tumor was easily separable from the surrounding bone, which suggests that it was encapsulated. In four cases no macroscopical cysts were present, the tumors being solid

throughout. Most of the tumors described have been cystic. The cysts may be as large as a hen's egg and usually contain a clear, yellowish, slightly viscid or serous fluid in which cholesterin crystals may be present. Such cysts may give to the tumor an irregular surface. The presence of a bony framework in the tumor is mentioned in twelve cases. Perhaps this framework corresponds to the alveolar process which forms about normally developing teeth. In Case No. 13 fully developed teeth were present.

Of the microscopical appearance of these tumors the clearest description is that given by Kruse (6). He reports three cases representing different stages in the development of the enamel organ. In the individual cases, also, different stages are represented. In the first case the epithelial constituents consist of dendritically branching twigs composed of epithelial cells and forming solid masses, situated in a poorly vascularized stroma. The form and arrangement of the cells is similar to that of the dental ridge in an early stage of development. The tumor therefore corresponds in its structure to an early stage of the "Anlage" of the tooth. Kruse's second case has in part the same structure as the first, but there is more tendency toward the formation of a peripheral layer of cylindrical cells. In some places small cysts are present and there is one macroscopical cyst 2 cm. in diameter. Comparison with a somewhat later stage of the dental "Anlage," where outer and inner enamel epithelium, stratum intermedium and stratum mucosum are present, show, according to the description, that the epithelial twigs of the tumor are in all details like the dental "Anlage," and that the relation of the cells to each other is the same. The third tumor is conspicuously cystic. Some cysts are microscopical in size while the largest is the size of a hen's egg. But the solid parts are microscopically like the first two tumors, presenting solid twigs of polygonal epithelial cells, some with a peripheral layer of cylindrical cells, some with beginning cyst-formation. The tumor consists largely of w ll developed cysts. The size and the structure of the cysts vary, but in general a definite size corresponds to a definite arrangement of the cells. In the smaller

cysts the wall is lined with low cylindrical epithelium, while the lumen contains a granular hyaline material. The larger cysts are lined by a more or less cubical epithelium and three to four layers of squamous cells. There is then, according to Kruse, a continuous series representing different degrees of differentiation, each of which has certain individual characters and in addition presents transitional stages to the others.

Chîbret's (2) work is especially valuable. He describes a case in which there is a pronounced tendency toward the formation of the various tissues of the teeth. The case presents all the stages in the formation of the tooth up to the development of enamel and of cemento-dental tissue. These substances are found at the borders of the most highly differentiated alveoli. The cemento-dental tissue resembles cement on the one hand, since it contains large osteoblasts, and dentine on the other hand, since branching canaliculi are present and vessels are wanting. In very few of the cases described in the literature does there appear to have been represented the epithelial sheath of Hertwig. Perhaps this explains the absence of roots and of characteristic cement and dentine.

A remarkable specimen is described by Hildebrand (7). He observed in the case of a boy nine years old an excessive development of masses of teeth in the interior of the upper and lower jaw bones on both sides. Not only conglomerations of teeth, but also more or less completely isolated teeth were present. The eruption of the teeth appeared to have been entirely irregular. Perhaps the entire epithelial "Anlage" of the teeth had assumed an abnormal function. In the soft tissues "Anlagen" of teeth in all stages of development were represented, together with dendritically branching masses of epithelium. These masses of epithelium are not described in detail, but if they are similar to those in the cases previously cited, the case perhaps belongs to the group under consideration.

The growth of such tumors apparently takes place by the formation of new epithelial twigs from those already present. The microscopical appearances are not always entirely typical. Some areas

may be extremely suggestive of carcinoma, the alveoli being irregular and containing epithelial cells that are only slightly differentiated. Structures closely resembling epithelial pearls are described. Bennecke (9) has described an invasion of the epithelial masses by vascular processes of the surrounding connective tissue. He believes that this process corresponds to a similar one that takes place in the normal enamel organ. The adamantine epitheliomata are relatively benign. I find no instance in which metastases have occurred. Enlargement of the lymph nodes may result from associated inflammatory processes. In one case a diagnosis of abscess was made and the tumor was not recognized until the abscess was opened.

The name "Adamantinoma" is suggested by Borst (4) for these tumors. The term Epithelioma adamantinum is perhaps more accurately descriptive. Where the cystic quality is pronounced the adjective "cysticum" may be added to the name as proposed by Goebel(3).

In some of the cases already cited there has been a history of trauma, such as a blow upon the jaw. Not infrequently it is said that an inflammatory process in connection with a tooth has been present at the onset. With the growth of the tumor there is pain and swelling, although pain is sometimes stated to be absent. Enlargement of the lymph nodes is mentioned only in infected cases and in four cases was said to have been absent. The absence of ulceration of the mucous membrane is emphasized in seven cases. Its presence is mentioned in only two cases and was apparently due to operative procedures or to inflammatory processes in connection with teeth. In the most careful descriptions it is insisted that the tumors have no connection with the epithelium of the mucous membrane. In the region of the tumor the teeth become more or less irregular and to a considerable extent are lost. In Case No. 13 the development of the teeth is said to have been irregular. Fluctuation could be demonstrated in one case. Its demonstration depends upon the presence of a fairly large cyst near the surface of the tumor. In Case No. 13 the teeth in the tumor could be palpated. The presence of parchment-like crepitation is mentioned in five cases, but could per-

Reference.	Sex and Age.	Duration.	Chief Symptoms.	Situation.	Character of Tumor.	Operation.	Result.
1. Albarran. Soc. de biol., 1887, 618, 667.	Male, 63 years.	(Recurrence) 8 years.	Tumor in mouth.	Left maxilla in region of second molar; growing into sinus.	In large part solid with two cysts; bony framework; fibrous and "mucoid" stroma.	Three incisions in 10 years; extirpation; resection.	Recurrence; second recurrence after 18 years; subsequent history unknown.
2. Becker (I). Arch. f. klin. Chir., 1894, xlvii, 52.	Female, 41 years.	3 years.	"Dental periostitis" at onset; infected.	Left; mandibula from incisors to angle.	Irregular surface.	Incision evacuating pus; part of alveolar process removed.	Recurrence after three years.
		(Recurrence) 1½ years.			Recurrence 4 cm. in diameter; contains cysts with clear serous fluid; fibrous stroma containing bone.	Resection of left half of mandibula.	Recovery; No recurrence at end of one year.
3. Ibid. (II).	Female, 26 years.	5 years.	Many teeth destroyed; fluctuation; crepitation.	Left angle of mandibula.	One large cyst with thick, solid wall; contains yellowish fluid.	Two incisions in mouth; external incision with removal of cyst wall.	No recurrence after 2½ years.
4. Ibid. (III).	.....	.....	.....	.....	Microscopically resembles No. 3.	.....	.....
5. Ibid. (IV).	Male, 39 years.	6 months.	Teeth destroyed; fistula in mouth.	Molar region and ramus mandibula on left side	....	Incision partly removing wall of cyst.	Recurrence after six months.
					Recurrence contains one large cyst, containing serous fluid; epithelial pearl-like structures.	Resection of portion mandibula involved.	No recurrence six months after resection.
6. Bennecke (I).	Adult.	....	Teeth about tumor few and irregular.	Median part of mandibula, extending farthest to left.	Nodular, 6 cm. in diameter; thin covering of bone; bony framework; numerous large cysts.	Resection.	Not stated.
7. Ibid. (II).	Adult.	.....	Teeth made irregular and loose.	Median part of mandibula.	Thin shell of bone; one small cyst. Cellular stroma but little stratum mucosum. 5 cm. in diameter.	Resection.	Not stated.
8. Borst. Die Lehre von den Geschwülsten, ii, 605.	....	.....	.....	....	Cystic; epithelial pearl-like structures without cornification.	....	....

Reference.	Sex and Age.	Duration.	Chief Symptoms.	Situation.	Character of Tumor.	Operation.	Result.
9. Chibret. Arch. de méd. expér., 1894, vi, 278.	Male, 53 years.	.....	Teeth made irregular; pain; crepitation; ulceration of mucous membrane.	Right; mandibula	Large as head of a foetus; cystic; cemento-dental tissue; enamel.	Resection of right half of mandibula.	Recovery; no recurrence after two years.
10. Derujinsky. Wiener klin. Wochenschr., 1890, iii, 775, 795.	Female, 25 years.	4 years.	Crepitation; ulceration of mucous membrane; probably secondary to operation; infection; enlarged cervical lymph nodes.	Left angle and ramus mandibula	Cystic and solid.	Two years ago removal of tumor ("Sarcoma").	Recurrence.
						Resection of left half of mandibula.	Not stated.
11. Haasler. Arch. f. klin. Chir., 1896, liii, 749.	Female, 64 years.	6 years.	Onset with dentalabscess; teeth over tumor lost; crepitation.	Median part of mandibula, extending farthest to right.	Cysts filled with pale yellow fluid, slightly viscid containing cholesterin crystals; bony framework; fibrous stroma.	Resection.	Two years without recurrence.
12. Hammer. Virchow's Archiv, cxlii, 503.	Male.	6 years.	.....	Left maxilla.	One large cyst with many smaller; dense, fibrous stroma with bony framework.	Resection of part of left maxilla.	Ten years without recurrence.
13. Hildebrand. Zeitschr. f. Chir., 1890, xxxi, 282; 1893, xxxv.	Male, 9 years.	1 year.	Teeth irregular and more numerous than normal; enlargement of maxillae and both sides of mandibula; teeth palpable through thinned bone.	Mandibula on right side from angle to median line; left side; both sinuses involved.	Sinus on each side filled with masses of developing teeth; about 200 teeth in all. Masses of epithelial cells branching dendritically.	Incision after six months.	
						External incision into both sinuses; apparently incomplete removal.	
						One year later incision on both sides of mandibula.	Recurrence on both sides of mandibula and in right maxilla one and a half years later.
						Repeated.	
14. Kruse (I). Virchow's Archiv, 1891, cxxiv, 137.	Male, 21 years.	10 years.	Dental abscess at onset; varying rate of growth; infected cyst; teeth absent in part over tumor.	Mandibula on right side from 2 cm. below coronoid process to canine tooth.	Nodular; easily separable from bone; microscopic cysts; stroma with bony framework; thin bony shell.	Resection of right half of mandibula.	Recovery.

Reference.	Sex and Age.	Duration.	Chief Symptoms.	Situation.	Character of Tumor.	Operation.	Result.
15. Ibid. (II).	Female, 12 years.	1 year.	Onset with toothache and swelling of bone; teeth in part absent.	Mandibula on right side from 3 mm. below articulation to canine.	Nodular; greatest diameter 8.2 cm.; cyst 2 cm. in diameter; stroma cellular with bony framework; bony shell.	Resection of right half of mandibula.	Recovery.
16. Ibid. (III).	Female, 30 years.	18 years.	Abscess at onset; irregular rate of growth; infection; fistula. Teeth absent; crepitation.	Mandibula on right side from articular surface nearly to median line.	Greatest diameter 13 cm.; nodular, cysts containing tenacious fluid; bony shell.	Resection of right half of mandibula.	Recovery.
7. Malassez (I).	Male, 11 years.	2 years.	Not stated.	Median part of mandibula.	Bony shell and framework; no cysts.	Resection.	Not stated.
18. Ibid. (II).	Male, 60 years.	23 years.	Irregular rate of growth; mucous membrane not connected with tumor.	Entire right body of mandibula.	Thin shell of bone; greatest diameter 8 cm.; many large cysts; bony framework; epithelial pearl-like structures; irregular masses of undifferentiated cells.	Not stated.	Not stated.
19. Monguidi. Correspondenzblatt f. Zahnärzte, 1895, xxiv, 17.	Female, 10 years.	1 year.	Swelling of cheek.	Right side of mandibula.	Thin shell of bone which is easily separated.	Excised several times.	Recurrence.
						Resection of right half of mandibula.	Not stated.
20. Nasse. Centralbl. f. Chir., 1890, xvii, Beilage zu No. 25, p. 33.	Female, 41 years.	10 years.	.....	Left side of mandibula.	Numerous small cysts; bony framework.	Resection of left half of mandibula.	Not stated.
21. Prym. Virchow's Archiv, 1897, cxlviii, 93.	Female, 31 years.	6 months.	Pain; swelling.	Right side of mandibula from angle to 3 cm. from spina mentalis and to coronoid process.	Cysts only microscopical; bony framework; cellular, nonvascular stroma.	Resection of right half of mandibula.	Not stated.
22. Steensland.	Female, 30 years.	2 years.	Swelling and pain; roots of teeth absorbed.	Left side of mandibula.	Diameter 7 cm.; thin shell of bone; cystic; stroma fibrous; dense, bony framework.	Resection.	No recurrence after 6 months.

haps have been demonstrated in more as the tumors were all surrounded by a thin shell of bone. In five cases the tumor was infected. There was present either an abscess or a fistula leading into the mouth. One may readily understand how a cyst might become infected as a result of incisions or inflammatory processes in connection with teeth. In three cases variation in the rate of growth of the tumor is mentioned.

BIBLIOGRAPHY.

1. Malassez. Sur l'existence d'amas épithéliaux autour de la racine des dents chez l'homme adulte et à l'état normal (débris épithéliaux paradentaires). *Arch. de physiol. norm. et path.*, 1885, v, 129.

Sur le rôle des débris épithéliaux paradentaires. *Arch. des physiol.*, 1885, v, 309.

2. Chîbret. Etude anatomo-pathologique d'un cas d'épithélioma adamantin. *Arch. de méd. expér.*, 1894, vi, 278.

3. Goebel. Ueber Kiefertumoren, deren Entstehung auf das Zahnsystem zurückzuführen ist. Zusammenfassendes Referat neuerer Arbeiten. *Centralblatt f. allg. Path. u. path. Anat.*, 1897, viii, 128 and 367.

4. Borst. Die Lehre von den Geschwülsten. 1902, ii, 605.

5. Becker. Zur Lehre von den gutartigen centralen Epithelialgeschwülsten der Kieferknochen. *Archiv f. klin. Chir.*, 1894, xlvii, 52.

6. Kruse. Ueber die Entwicklung cystischer Geschwülste im Unterkiefer. *Virchow's Archiv*, 1891, cxxiv, 137.

7. Hildebrand. Beitrag zur Lehre von den durch abnorme Zahnentwicklung bedingten Kieferformen. *Deutsche Zeitschr. für Chir.*, 1890-91, xxxi, 282; 1893, xxxv.

8. Massin. Ein Fall von angeborenem Epitheliom, entstanden aus dem Schmelzorgan. *Virchow's Archiv*, 1894, cxxxvi, 328.

9. Bennecke. Beitrag zur Kenntniss der centralen epithelialen Kiefergeschwülste. *Deutsche Zeitschr. f. Chir.*, 1896, xlii, 429.

DESCRIPTION OF PLATE.

Fig. 1.—Showing part of an alveolus.

a. External layer of cubical and cylindrical cells, which is in places invaginated and therefore appears to be within the alveolus.

b. Layer of cells corresponding to stratum intermedium.

c. Tissue formed by anastomosing stellate cells, corresponding to stratum mucosum.

d. Invaginated stroma.

e. Blood vessels in invaginated stroma.

Fig. 2.—*a*. Stroma.

b. Blood vessels in stroma.

c. Cells representing that stage which precedes the formation of the stratum intermedium and stratum mucosum.

d. Cells representing the early development of the stratum intermedium. Nuclei are deeply stained and have a ragged surface suggesting karyokinesis.

Fig. 1

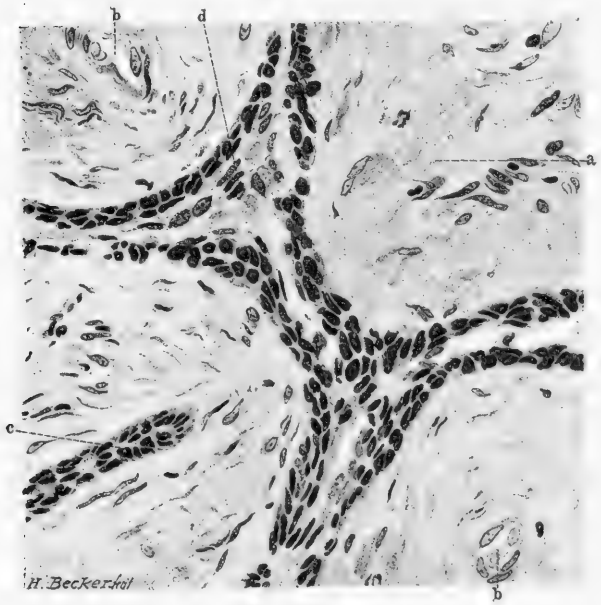

Fig 2

THE BACTERIOLOGY OF BRONCHO- AND LOBULAR PNEUMONIA IN INFANCY.

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The following study of the bacteriology of pneumonia in infancy was carried on at The Babies' Hospital for several years, with the view of determining the relationship between the extent of the pneumonic areas and the variety of bacteria present, and the difference, if any, between the bacteriology of the primary and of the secondary infections.

TECHNIQUE.

Forceps and scalpels were enclosed in sheet iron boxes and sterilized in the hot-air oven for one hour at 120° C.; of these several were kept ready for use. The sternum having been removed with a sterile knife, the lungs were in turn quickly drawn up with forceps, sufficiently to expose the consolidated areas. Incisions or stabs were made into these, a fresh knife being used for each stab. The platinum loop was then plunged into the incision and glucose agar slants inoculated. Cover-glasses were also spread and served as a control. After eighteen to twenty-four hours in the thermostat the tubes were examined and plates prepared from them. All the cultures of pneumococci and many of those of streptococci were inoculated into white mice and rabbits. Cultures from the heart's blood and from the other viscera of the animals which succumbed were made at the autopsy in the same manner as from the human cases. Avoiding the searing of the organs in cases from which the pneumococcus is to be cultivated proved an advantage. Microscopic examination of the lungs was made in every case.

AGE AND SEX.

The one hundred cases ranged from eighteen days to three and a half years of age.

13 were under 3 months old.
26 " between 3 and 6 months old.
30 " " 6 and 12 " "
20 " " 12 and 18 " "
6 " " 18 and 24 " "
4 " " 2 and 3 years old.
1 was 3½ years old.

Among these were 56 males and 44 females.

PRIMARY BRONCHO-PNEUMONIA.

The series includes 33 primary cases, aged from six weeks to two and a half years. In eleven cases the consolidated areas were relatively small and scattered in both lungs; in six they existed in one lung alone. The areas had coalesced, so that the greater part of one lobe was involved in two cases and of two lobes in the same number. In twelve cases the greater part of a lobe was solid while smaller areas were scattered throughout other lobes.

The pneumococcus was present in pure cultures in fifteen, associated with the streptococcus in seven, and the staphylococcus pyogenes aureus in three cases. The streptococcus was found alone in two cases and associated with the staphylococcus pyogenes aureus in two more. The staphylococcus pyogenes aureus was present alone in two cases, associated with the bacillus coli communis in one case, and the streptococcus and oidium albicans in one case.

The following table shows the anatomical distribution of the lesions, the bacteriology, and the condition of the pleura in the primary cases.

Pneumonic Areas.		Pleura.			Pus.				Bacteriology.
Scattered.	All or greater part of one or more lobes.	Normal.	Covered with fibrin.	Covered with pus.	In Bronchi.	Pleura.	Pulmonary Abscesses.	Purulent Infiltration.	
—		—		—	—	—	—		Pneumococcus.
—			—	—	—	—	—	—	" and streptococcus.
—			—	—	—	—	—	—	" " staph. pyog. aureus.
—		—		—	—	—	—	—	Staphylococcus pyogenes aureus.
	—	—	—		—		—	—	Pneumococcus.
	—	—	—		—		—		"
	—	—	—		—		—	—	"
	—	—	—		—		—	—	"
	—	—		—	—	—	—	—	"
	—	—		—	—	—	—	—	"
	—	—		—	—	—	—	—	"
	—		—	—	—	—	—	—	"
	—	—			—		—		" and streptococcus.
	—	—		—	—	—	—	—	" " "
	—	—		—	—	—	—	—	" " staph. p. aureus.
	—		—	—		—	—	—	" " " " "
	—	—		—	—	—	—	—	Staphylococcus pyogenes aureus.
	—		—	—	—	—	—	—	Streptococ. and staph. pyog. aureus.
	—	—	—		—		—	—	" " " " "
	—	—		—	—	—	—	—	" , s. p. aureus, soor.
	—		—	—	—	—	—	—	Staphylococcus p. aureus, b. coli com.
			—	—	—	—		—	Pneumococcus.
			—	—	—	—	—		"
			—	—	—	—	—	—	"
		—		—	—	—	—	—	"
		—		—	—	—	—	—	"
		—	—				—	—	"
		—						—	" and streptococcus.
		—		—	—	—	—	—	" " "
		—	—		—		—	—	" " "
			—	—	—	—	—	—	" " "
			—	—		—	—	—	Streptococcus.
			—	—	—	—		—	"

—means none.

The relatively large percentage of cases in which the pneumococcus was found, alone or in association with other organisms, may be

attributed to three factors: the short time elapsing between death and autopsy, the use of glucose glycerine agar for the first inoculations, and the avoidance of heating the lung before taking cultures from it.

While the pneumococcus was present in 81 per cent of the cases in which all or the greater part of one lobe was involved, being alone in 41 per cent, it was found in 71 per cent of the focalized cases, in 47 per cent being in pure culture. It would seem, therefore, that in the present primary series the pneumococcus was present in a somewhat larger percentage of cases of broncho-pneumonia involving the greater part of one or more lobes, as compared with those cases in which the pneumonic areas were merely scattered throughout one or both lungs. In the fifteen cases due to the pneumococcus alone, there was acute fibrinous pleurisy present in six, acute purulent exudate in five, and a normal pleura in four. Of the twenty-five cases in which the pneumococcus was present, eight presented no lesion of the pleura. Both cases in which the streptococcus was found in pure culture had a healthy pleura. There was a fibrinous pleurisy in both of the cases due to the staphylococcus pyogenes aureus. Three cases were complicated by inflammation of one or more other serous membranes (pericardium, peritoneum, pia mater, synovial membranes) from all of which the pneumococcus was cultivated. One case, due to the streptococcus and the staphylococcus pyogenes aureus, was followed by empyema and osteo-myelitis of one rib. Two cases showed the presence of true lobar pneumonia as well as broncho-pneumonia, and the pneumococcus was present in both lesions.

SECONDARY BRONCHO- AND LOBULAR PNEUMONIA.

I. Secondary to Athrepsia. 20 cases, 17 having been diagnosed before death. In 10 cases the pneumonic areas involved all or the greater part of one or more lobes, and in 10 they were scattered throughout one or both lungs.

The pneumococcus was present in pure culture in 5 cases, together with the staphylococcus pyogenes aureus in 5 cases, the staphylo-

coccus pyogenes albus and with the bacillus coli communis each in one case. The streptococcus was found in pure culture in one case, together with the bacillus coli communis in two cases, the staphylococcus pyogenes aureus and the staphylococcus pyogenes albus each in one case. The staphylococcus pyogenes aureus was found in pure cultures in two cases. The bacillus lactis aerogenes and oidium albicans were found in one case. There was fibrinous pleurisy in four cases, two of which were accompanied by purulent bronchitis and one by purulent infiltration of the lung as well. In the latter case the pneumococcus and the staphylococcus pyogenes aureus were found; in the former, the streptococcus and the bacillus coli communis, which combination of organisms was also present in one other case, while the pneumococcus alone caused the fourth one.

II. Enterocolitis. Among these three cases, all having given symptoms of broncho-pneumonia before death, one was complicated by furunculosis. In this latter case both the pneumococcus and the streptococcus were cultivated from the lungs. Among the rest the pneumococcus was found in pure culture in one case, and the bacillus coli communis and the bacillus proteus vulgaris were grown from the third case. This last case showed scattered pneumonic areas in the right upper lobe only, while in the pneumococcus case the entire left upper lobe was solid and in the other there were focalized areas in both lungs and consolidation of the lingula.

III. Diphtheria. Five cases were studied. The pneumococcus was found in pure culture in one case, the streptococcus in two cases, and the Klebs-Loeffler bacillus in one case. Finally in one case the streptococcus and the Klebs-Loeffler bacillus were present not only in the lungs, but in the kidneys as well. In the last one there had been no symptoms of nephritis during life. Laryngeal diphtheria existed in all the cases, while in three the pseudo-membrane had extended into the trachea, although it was never present in the bronchi.

IV. Measles. Three cases, in two of which the pneumococcus was found in pure culture, were examined. Both showed scattered

foci of consolidation. In one there was a very recent fibrinous pleurisy and gangrenous areas in both lower lobes. In the third case empyema, purulent pericarditis, and small pulmonary abscesses developed, the streptococcus alone being found. It had also been cultivated from the empyemic pus before death in this case.

V. Meningitis. Two cases were studied in which one involved the cerebral membranes only. The pneumococcus and the streptococcus were grown from the lungs, which contained scattered pneumonic areas and consolidation of three-quarters of the right lower lobe, and showed fibrino-purulent pleurisy. The pneumococcus was present alone in the meningeal pus and in the kidneys. The second case followed spina bifida. From the scattered pneumonic areas in both lungs in this case the streptococcus and oidium albicans were grown. The streptococcus in pure culture was found in the pus of the cerebro-spinal meninges, but the soor fungus appeared in the cultures made from both kidneys. Thrush had been present in the mouth, but had disappeared before death.

VI. Cerebral abscess. One case occurred in which the terminal pneumonia was due to a mixture of the pneumococcus and colon bacillus.

VII. Malaria. Two cases in which death was caused by bronchopneumonia. The consolidated foci were scattered throughout both lungs. The staphylococcus pyogenes aureus was cultivated from one case and the bacillus lactis aerogenes from the other. Tertian malarial parasites were found in sections of the lungs and spleen in both cases and of the kidneys in one case.

VIII. Septicaemia. Six cases were studied. In two the pneumococcus and the bacillus pyocyaneus were found in the lungs, the bacillus being present in pure culture in the other viscera as well. The streptococcus alone was found in a case following a surgical operation. The streptococcus and the staphylococcus pyogenes aureus were present in one case in which the sepsis resulted from erysipelas originating in a cervical wound. The streptococcus alone was found in the heart's blood and kidneys. Staphylococcus pyogenes aureus

in pure culture appeared in two cases, one of which followed furunculosis, the same organism having been cultivated from several furuncles during life.

IX. Tuberculosis. Twenty-five cases were studied. Eight of these were of the pneumonic type, running a clinical course like that of acute broncho-pneumonia, the tuberculosis having been wholly unsuspected in three instances, in two of which the tubercles were found to be very recent and scattered; in another case there was a cavity in the right middle lobe. In all but two of these eight cases there was consolidation of one or more lobes, accompanied by acute fibrinous pleurisy in three instances and by purulent pleurisy in one instance.

The pneumococcus was found alone in two cases, in association with the streptococcus and with the staphylococcus pyogenes aureus in two cases each. The streptococcus and the staphylococcus pyogenes aureus were combined in two cases. Tubercle bacilli were found in smears and in sections of the tissue in every instance.

In one child the clinical course was that of athrepsia, the child dying of broncho-pneumonia. At autopsy the lungs contained recent miliary tubercles and scattered areas of broncho-pneumonia, from the latter of which the pneumococcus was grown in pure culture.

Sixteen cases ran a clinical course of general miliary tuberculosis. The lungs showed consolidation of all or nearly all of either one or two lobes in seven cases, in three of which the pneumococcus was present in pure culture. In one case of the focal variety it was also found pure. The pneumococcus and the streptococcus were found in four cases and the pneumococcus and staphylococcus pyogenes aureus in three. The streptococcus was alone in three, the staphylococcus pyogenes aureus in two instances.

In seven of the twenty-five cases smaller or larger cavities were present (cavities of the right lung occurred five times, of the left lung once, of both lungs once). Among these 7 cases the pneumococcus was found in pure culture twice, associated with the streptococcus twice, and with the staphylococcus pyogenes aureus twice. The streptococcus was present in pure culture in one case. In one case of empyema the pus contained tubercle bacilli and streptococci.

Summarizing the above results we find that the pneumococcus was present in 67 of the 100 cases examined. The positive cases can be divided into 25 primary and 42 secondary pneumonias. While the pneumococcus appeared in 76 per cent of the primary cases, it occurred in only 63 per cent of the secondary cases. And while it was present in pure culture in 42 per cent of the primary cases, it was found alone in only 15 per cent of the secondary cases. The following table summarizes the bacteriology of the entire series:

	Primary.	Secondary.	Total.	
Pneumococcus	14	10	24	
" and tubercle bacillus	0	7	7	
" " streptococcus	7	10	17	
" " staph. p. aureus	3	10	13	
" " diphtheria bacillus	0	1	1	67
" " bacillus pyocyaneus	0	2	2	
" " " coli com.	0	2	2	
" " staph. p. albus	0	1	1	
Streptococcus	2	6	8	
" " staph. p. aureus	2	3	5	
" " " " and soor	1	0	1	
" " diphtheria bacillus	0	1	1	
" " staph. p. albus	0	2	2	
" " bacillus coli com	0	1	1	
" " soor	0	2	2	33
Staphylococcus pyogenes aureus	3	6	9	
Staph. p. aureus and bacillus coli com	1	0	1	
Bacillus lactis aerogenes	0	1	1	
" " " and soor	0	1	1	
" coli com. and proteus	0	1	1	
	33	67	100	

The 67 cases showing the pneumococcus were accompanied by inflammation of the pleura in 51 per cent (34 cases), the lesion being an acute fibrinous exudate in 22 instances (13 secondary, 9 primary), acute purulent exudate (empyema) in 4 instances (2 primary and 2 secondary), and a fibrino-purulent one in 8 instances (2 primary, 6 secondary). The cases from which the pneumococcus was absent (33) showed pleurisy in one-third of the number (11 cases), the inflammation being of the acute fibrinous variety in 9 instances (3 primary and 6 secondary) and purulent in 2 instances (1 primary and 1 secondary). So that the pneumococcus cases showed a decidedly larger number of examples of pleurisy than did those of other organisms. The percentage of empyema cases were exactly the same (6%) in both varieties.

Purulent infiltrations of the lungs occurred in 7 cases, and all these contained the pneumococcus, either alone or combined with the streptococcus (3 instances) and the staphylococcus pyogenes aureus (1 instance).

Small pulmonary abscesses existed in 7 cases. In 5 of these the pneumococcus occurred, twice combined with the tubercle bacillus, once with B. pyocyaneus and once with the streptococcus. In two instances the streptococcus was present alone.

Of the 100 cases, 45 were coalescent, and therefore "pseudo-lobar" in distribution, with the pneumococcus present in 33 instances, or 73 per cent. In 31 of 55 focalized cases, or 56 per cent, the pneumococcus was also found—a decided decrease.

The cases of primary broncho-pneumonia in the present series show a larger percentage of pneumococcous infections than do the secondary cases. Those cases, both primary and secondary, in which the inflammation involves all or nearly all of a lobe, show a larger percentage of pneumococcus infections than do cases of the focal variety. The pneumococcus cases show a larger percentage of inflammation of the pleura, and a greater tendency to purulent infiltration and abscess formation.

Dürck[1] states that the pneumococcus was present in every case of primary lobar, pseudo-lobar or lobular pneumonia. While our own number of cases containing the pneumococcus is larger than that of Netter[2] and Neumann,[3] it is about the same as Mosny's,[4] whose primary cases, however, numbered only four. Queisner[5] found the pneumococcus in eight cases examined, while Blumer[6] did not find it at all in eighteen.

The secondary cases in the main bear out Pearce's[7] statement that

[1] *Deut. Archiv f. klin. Med.*, 1897, lviii, 368.

[2] *Arch. de med. exp.*, 1892, iv, 28.

[3] *Jahrb. f. Kinderheilk.*, 1899–90, xxx, 233.

[4] Etude sur la broncho-pneumonie, Paris, 1891.

[5] *Jahrb. f. Kinderheilk.*, 1899–90, xxx, 277.

[6] *Albany Med. Annals*, 1901, xxii, 424.

[7] *Boston Med. and Surg. Journ.*, 1897, cxxxvii, 561.

in all cases where a local or general infection existed the associated broncho-pneumonia was due to the same micro-organism. Blumer had the same experience. In the five athrepsia cases in which the pneumococcus was found in pure culture, no other inflammatory lesion was present. The same was true of the one case containing the streptococcus alone. Three cases having the staphylococcus pyogenes aureus in the lungs at autopsy (combined with the pneumococcus in two instances) had shown the same organism in the discharge from a purulent otitis media before death.

Since the influenza bacillus was not searched for as a matter of routine, it may, of course, have been overlooked in some instances. In two cases which came to autopsy with a diagnosis of influenza cultures from the lungs were made on blood agar with negative results.

A MALIGNANT TERATOMA OF THE PERINEUM.

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The clinical history of the case, for which I am indebted to Drs. W. H. Whitehead and I. H. Manning, of Rocky Mount, N. C., is as follows:

The patient was a male child, born in October, 1898. In July, 1899, the child's father noticed a small tumor, about the size of an acorn, situated in the middle line half way between the anus and scrotum. The tumor was subcutaneous, and neither painful nor tender. In October, 1900, it began to increase in size quite rapidly, as the result, the father thought, of several blows upon it; and the penis became persistently erect. The child had no other abnormality, and seemed in excellent physical condition. The growth was removed by Dr. Tiffany, of Baltimore, in February, 1901. There was a speedy recurrence *in loco,* so that under date of March 18, 1901, Dr. Manning wrote me that the site of the wound was occupied by a sloughing cauliflower mass as large as an orange, and that a lymph node in the right groin was the seat of a metastasis, the size of a walnut, ulcerating through the skin.

At the time of the operation two portions of the growth were sent in alcohol to me for examination. One of them, almond-shaped, and about 3 cm. in its longest diameter, consisted largely of bone. Pieces decalcified in 5% nitric acid showed the following structure. Both surfaces of the piece are covered by epidermis, which, though quite thin, can in most places be divided into the three layers characteristic of that structure, with here and there an epithelial peg. The epidermis rests upon a corium which contains rudimentary papillæ, sebaceous follicles, and hair-roots. A little deeper in the section are numerous plates of bone with rather large marrow spaces. In addition, there are found a number of cyst-like spaces lined by an epi-

dermis, which rests upon connective tissue containing sebaceous follicles and hair-roots. These spaces contain amorphous debris and a few hairs—in short, in the interior of the tumor are several minute dermoids. There is another space quite different from those just described. It is lined by mucous membrane, the epithelium of which is composed of stratified columnar ciliated cells. The epithelium presents a sinuous outline, being thrown up into minute ridges by a loose cellular submucosa. Outside of this is a quite distinct circular band of smooth muscle tissue. There is still another space lined by stratified squamous epithelium, which is more or less parallel with the preceding. Indeed, the two run together throughout a considerable extent of the piece, and at one point there is a wide opening between them, with quite a sharp line of demarkation between the two sorts of epithelium. In addition to these structures, there were noted a very few striped muscle cells, and a plate of cartilage which stained deeply with hæmatoxylin. The tumor is fairly well supplied with blood-vessels and lymphatics.

Microscopical examination of the second piece of tissue previously alluded to, shows it to consist of striped muscle tissue extensively infiltrated by masses of new growth. The muscle is, for the most part, in various stages of atrophy and degeneration. Presumably it is a portion of one of the perineal muscles. The tumor masses may be divided roughly, according to size, into large and small. The latter are more or less circular in shape. In some cases it can be made out quite clearly that they are contained within spaces lined by flattened endothelial cells quite distinct from the tumor cells; while in other cases there is no clear line of separation between them, the two passing into each other by a gradual transition. These spaces contain no blood, and are probably lymphatics. In the spaces the cells are cuboidal or spindle-shaped, and have large nuclei, of which some contain well-marked nucleoli, others only chromatic granules. In some of the spaces the arrangement of the cells is distinctly papillary in character: on a blood capillary as a stalk the cells are placed in a thickness of one or more layers; sometimes the

capillary can be traced to a vessel in the wall of the space. The larger masses possess no characteristic shape, and are evidently the result of extensive infiltration of the muscle. It is somewhat remarkable to find the muscle fibers resisting the attack to the extent that frequently muscle fibers, though more or less degenerated, are found within large masses of tumor cells. In these masses the cells are inclined to be spindle-shaped; they are often packed closely together and arranged in columns, separated from one another solely by blood capillaries, which, indeed, constitute the only stroma that the growth possesses. Transitions between the two sets of tumor masses are abundant. A striking feature in both categories is furnished by spaces circular on cross-section and of various sizes, which contain only amorphous granular material and leucocytes. These seem to be due, in some instances at least, to necrosis, in one situation of tumor cells, in another of muscle fibers. In either case the resulting cavity is not filled by tumor cells; on the contrary, the spaces seem permanent, and are lined by tumor cells.

The clinical history of the case and the microscopical examination of the specimens make it certain that we are here dealing with a congenital tumor of a teratoid nature, which has given rise to a malignant neoplasm. Before attempting to proceed to an exact diagnosis let us summarize the leading features. The tumor was found to contain several minute dermoid cysts, plates of bone, and two structures which are susceptible of interpretation as fœtal organs in an exceedingly rudimentary condition, viz., a cylindrical cavity lined by mucous membrane, whose epithelial cells were stratified, columnar and ciliated, surrounded by unstriated muscle tissue; and another cavity communicating with the above, but lined by stratified squamous epithelium. Nothing was seen which could be looked upon as representing the central nervous system. The location of the tumor exactly in the center of the perineum should also be borne in mind.

When we come to consider its exact position among such tumors we are met first of all by the difficulties which arise from the lack of a uniform nomenclature. The classification proposed by the late

Birch-Hirschfeld[1] is commendable by reason of its simplicity. He divides these tumors into the following classes (omitting those congenital mixed tumors which develop from the anlages of the Wolffian body):

A. Tumors developed as the result of invagination and snaring off of portion of the ectoderm or endoderm, or of either of these layers along with mesoderm—dermoid cysts and the so-called entero-cysts.

B. Tumors developed in the same way as above, except that all three layers of the blastoderm are involved. These he calls "teratoids." They contain representatives of all of the germ-layers, but no distinct organs. It might be added that a tumor to be placed in this class should be easily referable to the fœtal anlages in its vicinity.

C. Tumors which are really rudimentary embryos. These are the true teratomata, and may be divided into two varieties: the autochthonous, or the teratomata of the genital glands; and heterochthonous, or those which result from the inclusion of one embryo by another—the cases of *foetus in foetu.*

Following this classification, it seems clear that our tumor belongs either to Class B or to Class C. Birch-Hirschfeld himself, in discussing this classification, admitted that the division between the two classes was more or less artificial, and thought that it might be very difficult to distinguish between them in some cases. It may be said that the present tumor offers such a case. No assistance is to be obtained from a study of similar cases; for, as far as I can learn from an examination of the literature, all the tumors of a teratoid nature which have been found in the perineum belong to Class A of Birch-Hirschfeld's classification, that is, were either dermoids or entero-cysts referable to the changes which occur in the cloaca and urogenital sinus during the development of the external genitals. It is true that in the region dorsal to the rectum numerous teratoid tumors have been discovered, which were quite complex in structure, and which, as a rule, were susceptible of explanation by the theory of invagination at the caudal extremity of the embryo—a region

[1] Allg. patholog. Anat., Leipzig, 1897.

which is remarkable because of the existence of the cloaca and neurenteric canal in addition to a considerable mass of undifferentiated cells. The neurenteric canal in particular is invoked to explain the occurrence of columnar ciliated epithelium in such tumors. It is difficult to understand, however, how such invaginations could produce teratoids anterior to the rectum; and, as has been said, none have been described in that region. It is conceivable, though, that such a tumor as the one under consideration might arise from a partial snaring off of a large portion of the caudal extremity of the embryo at a very early stage, the separated part being included by a regenerated caudal extremity. Such a separated portion would contain anlages to which the various structures contained in the tumor might be referred. The cavity lined by stratified squamous epithelium might be considered as derived from the ectodermal plate which closes the cloaca, and the other cavity might be considered as a representative of the cloaca or, more likely, of one of the genital ducts. The fact that such a great variety of epithelial cells is found in different regions of the urogenital tract, would make it impossible absolutely to deny that the stratified columnar ciliated epithelium found in the tumor originated in that tract. It is a fact, too, that portions of the malignant growth remind one forcibly of the appearances in the fimbriated extremity of the Falloppian tube. It is not impossible that the malignant growth took its origin in the lining of a rudimentary Müller's duct—in which event we might term it a mesothelioma, as the mesothelium furnishes the lining of this duct. The fact that no evidences of a central nervous system were found is not a valid objection to the above theory, as all of the tumor was not at my disposal.

On the whole it would seem simpler to regard this tumor as a true teratoma in the sense employed in Birch-Hirschfeld's classification. If we adopt this view the question of its genesis next arises. The parthogenetic hypothesis, which has received new force from the brilliant experiments of Loeb, could not obtain here, however applicable it may be to teratoma of the genital glands. Accordingly we should have to conclude that the tumor is a true *foetus in*

foetu in a very rudimentary condition, and that the avenue of inclusion was the urogenital sinus, the closure of the genital folds completely covering it in. Under this interpretation the rudimentary organs referred to would represent the anterior portion of the alimentary canal and the respiratory tract, in accordance with the well-known fact that in teratoma the cephalic extremity receives most development. The absence of brain may be explained by the fact that only a portion of the tumor could be examined. However, brain is not found in all teratomata.

That a mass composed of such tissues should give rise to malignant growth does not seem surprising; rather the wonder is that malignant growths do not arise more frequently in connection with teratomata. Montgomery[2] has recently examined the literature with reference to this point, and, omitting certain tumors of the ovary whose nature was obscure, he could collect but ten cases of malignant teratomata.

In the present case a diligent search was made to determine from what tissue of the teratoma the malignant growth took its origin; but nothing was found to shed any light upon this question—possibly because not all of the tumor was at my disposal. The diagnosis, therefore, has to be made as far as possible from the histological structure alone. It would seem most in accordance with this structure to regard the tumor as an endothelioma which, taking its origin in the endothelium of the lymphatics, developed in and along these spaces finally to break through and infiltrate the tissues of the perineum diffusely.

EXPLANATION OF PLATES.

PLATE XXVIII.

Fig. 1. Section through middle of the teratoma, *A* small dermoid cyst, *B* cavity lined by stratified squamous epithelium, *C* cavity lined by stratified columnar ciliated epithelium, *D* smooth muscle tissue.

Fig. 2. Tumor masses in lymphatics.

PLATE XXIX.

Fig. 3. The same under higher magnification.

Fig. 4. Through the malignant portion of the tumor.

[2] *Journal of Experimental Medicine*, 1898, iii, 259.

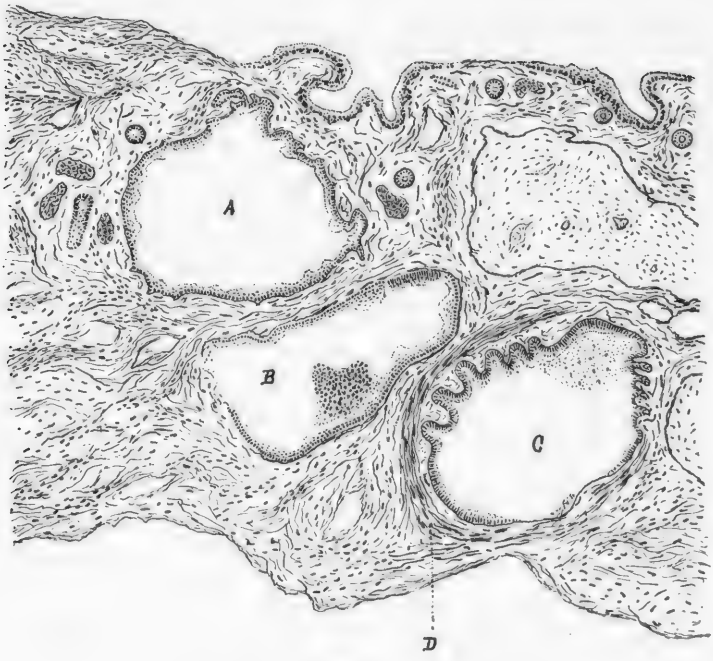

FIG. 1.

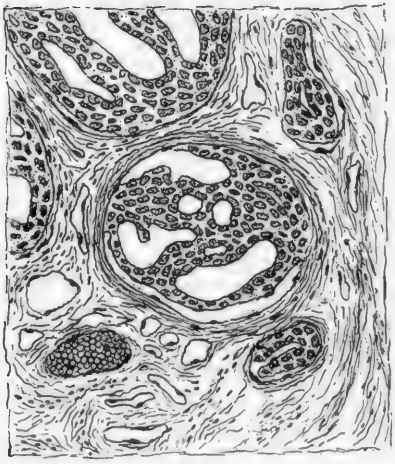

FIG. 2.

FIG. 3.

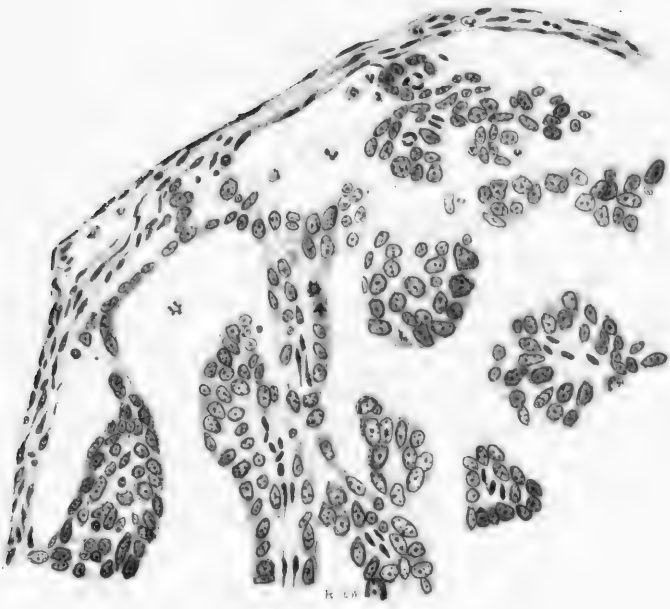

FIG. 4.

DERMATOMYOSITIS, WITH REPORT OF A CASE WHICH PRESENTED A RARE MUSCLE ANOMALY BUT ONCE DESCRIBED IN MAN.

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Inflammatory changes in muscles have long been recognized as occurring independently or in connection with certain diseases. Hunter (1) is said to have described cases of myositis in 1784 and to have noted the insusceptibility of muscular tissue to inflammation and its consequences. I have been unable to find any such reference in his writings. During the past century cases have been published in many different countries and a considerable amount of literature is to be found on this affection.

A multiple muscle inflammation, however, presenting also other well known symptoms, was not described as such until 1887. In that year E. Wagner (2) of Leipsic, Unverricht (3) of the Polyclinic at Jena, and Hepp (4) of Kussmaul's clinic at Strasburg, almost simultaneously reported cases. Though Wagner's case was first published, it was in reality the last seen, for Hepp made his observation two months previously and Unverricht's case had been noted seven years before either of the above two. The pathological findings, with a meager history of this last case, were presented by Marchand (5) to the Schlesische Gesellschaft für vaterländ. Cultur on October 22, 1880, and later appeared in the society's transactions and in the Breslauer ärztliche Zeitschrift of that same year. The case has caused a good deal of confusion among writers on this disease. Herrick (6), Jacoby (7), and others have described it as another case of an earlier observation, while Lewy (8) thinks it is an additional one which Unverricht has later more fully reported. This error may be due to the fact that Unverricht in two of his articles

made the date of seeing the patient a year later than it should have been. Yet a careful examination will convince one that the same case is dealt with. Hepp's case was also reported by Henry Jackson (9) of Boston in 1887—a fact which seems to have been entirely overlooked.

Before these observations we find two others which, although representing cases rather meagerly described and otherwise diagnosed, should undoubtedly be considered as bearing on this disease. I refer to a case seen by E. Wagner (10) in 1863, and to a later one observed by Potain (11) in Paris. They were diagnosed at the time as instances of periostitis and an atypical form of glanders. Following the publication of the first three recognized instances Jacoby (12) and Löwenfeld (13) gave additional cases and discussed the previous papers on the subject. A little later Unverricht (14) presented the clinical history of another case, which differed from those previously reported in terminating favorably. The close association in this patient of dermatitis with a multiple muscle inflammation led Unverricht to coin the term *Dermatomyositis,* which has been frequently adopted to designate this affection. Boeck (15) and Strümpell (16) subsequently published other cases, the latter giving an excellent account of all that was known of the disease.

In the following years many German writers reported so-called cases which were subjected to a critical examination in 1896 by R. Pfeiffer (17), and the histories given by Schnell (18), Lewy (19) (three out of his four cases), Fraenkel (20), Buss (21), Herz (22), Albu (23), Schultze (24), and Schultzen (25) were, rightly we think, adjudged as atypical and belonging to another disease. The cases of v. Kornilow (26), Jollasse (27), and Thiele (28) can be set aside on the same grounds. Neubauer's case (29) more nearly resembles polymyositis haemorrhagica. During these years, however, Fuckel (30), Senator (31), Lewy (32), Köster (33), and Kell (34)[1] have given us undoubted examples of the disease, while Gowers

[1] Two of Kell's three cases have not been added to our list as they were of a doubtful abortive type and somewhat atypical in character.

(35) has reported a case which probably should not be classed here, as it was associated with a polyneuritis. More recently Frohmann (36), Bonnet (37), Lepine (38), Janowsky and Wyssokowicz (39), Bacialli (40), Oppenheim (41), and Christen (42) have added to the number already recorded. Forcheimer's case (43) I have also placed in this list, although it is not as typical as the others. It is interesting to note that one of Oppenheim's cases, reported four years ago as a typical instance of this affection, now presents all the symptoms of scleroderma. During the past two years Vincent (44), Schüller (45) and Jessen (46) have likewise published histories of patients with polymyositis, but none of them had the special type of disease which would entitle them to admission to our group. From time to time different writers have collected the previously existing cases but, with the exception of Lorenz (47) and R. Pfeiffer (48), they appear to have done so without any discrimination. Lewy was able to find twenty-one cases in 1893, while Köster, three years later, added two more to that number. Both of these writers, however, included atypical cases and those of a closely allied affection—polymyositis haemorrhagica.

We have found, including our own, twenty-eight quite typical histories, which are classified according to countries as follows: Germany, 19; France, 2; Sweden, 2; Italy, 1; Cuba, 1; United States, 3; total 28.

Gowers' case is the only one as yet observed in England. In Italy two cases of polymyositis haemorrhagica have been described and one of dermatomyositis, while, though several writers have writen on this affection, the only case yet published in Russia (49)[2] belongs to another type of myositis.

Dermatomyositis may be defined as an acute, subacute, or chronic disease of unknown origin, characterized by a gradual onset with vague and indefinite prodromata, followed by oedema, dermatitis, and a multiple muscle inflammation.

[2] I desire to thank Dr. Thayer for translating this case from the Russian for me.

Etiology.—On this point we are as much in the dark as were the three writers who first described the disease. Hepp and others have spoken strongly in favor of its being due to an infection and the presence of a splenic tumor, fever, and angina, seem to support this view. From Senator's time the following three theories as to its origin have been advanced:

I. It is due to a specific microorganism (vegetable parasite). This view is gradually losing ground. Bacteria have been frequently sought for in the inflammatory oedema and in the tissues, with negative results in every instance save one (Bacialli's case), and cases of this disease reported as being due to certain bacteria have been shown to belong to another class of muscle inflammation. Bauer (50) and Georgievski (51) have recently reported cases of the allied affection—polymyositis haemorrhagica—which were probably due to Staphylococcus pyogenes aureus and Staphylococcus pyogenes albus, obtained in pure cultures from the tissues. In Bacialli's case, however, the Staphylococcus pyogenes albus was said to be very much attenuated in virulence. His findings, as well as Georgievski's, can hardly be considered as conclusive in view of the universal presence of Staphylococcus albus in the deep layers of the skin (Thayer).

II. It is due to an animal parasite. This idea was first advanced by Unverricht, who thought the parasite might belong to the gregarinae. In support of this theory L. Pfeiffer (52) states that the muscle findings in this disease are similar to those seen in the muscles of horses and dogs infected with gregarinae, and he says that Virchow (53) has mentioned cutaneous rashes in hogs that were similar to the changes in the skin occasionally found in dermatomyositis. The only instance, however, in which these parasites were found in man was reported in 1891 by Klebs (54), who observed them in a case of muscular atrophy, where they were at first taken for muscle nuclei. Negative results have attended the search for them in seven cases, in two of which L. Pfeiffer also examined the sections. This unsuccessful quest Pfeiffer would explain as due to the fact that the methods of preserving the tissues and preparing the specimens mili-

tate against a ready recognition of the parasite. They may be present but be overlooked, or, although they can cause a muscle inflammation by their presence or their toxin, the muscles containing them may escape microscopic examination.

III. It is due to a toxin. This view was given prominence by Senator, to whom it was suggested by his second case, which began with symptoms of gastro-intestinal irritation after the patient had eaten stale crabs. Kell's case is also somewhat similar, the symptoms appearing a few hours after the ingestion of fish. No gastro-intestinal symptoms were here noted. In addition to these, Boeck's case has been put in this group, inasmuch as the disease was observed after the energetic rubbing of copaiba balsam into the skin. Boeck, however, does not consider this point to have any etiological significance.

Köster (55) suggests that the symptoms may be due to a primary implication of the vascular system. On this assumption Lepine has proposed the term *Angiomyositis.* It is interesting to note in this connection that Rosenblatt (56) has described a case resembling dermatomyositis, which showed thrombus formation in the vessels, degenerative changes in the vessel wall with fibrin and leucocytes in and about the vessel wall.

Cold and fatigue are said to play a very minor part, though occasionally, as in our case, they seem to be exciting causes.

Distribution.—Cases have been reported, as has been already noted, in the United States and in a number of European countries.

Race.—The Anglo-Germanic has furnished most of the instances, followed by the Latin and Scandinavian. Our patient is the only case in the Negro yet reported.

Season.—The time of year seems to have no connection with the disease. Ten were attacked in the winter, five in the spring, eight in the summer and five in the autumn.

Sex.—The affection has been observed in seventeen males and eleven females. Its distribution among the sexes is probably about equal.

Age.—The disease is usually met with in middle life, though cases at both extremes are found, *e. g.,* Löwenfeld's and Fuckel's cases.

Morbid Anatomy.—Of the seventeen fatal cases autopsies have been performed in all but seven, and in four of these the excision of a piece of muscle was allowed. We have consequently a fair amount of data on which to base our conclusion as to the changes found in this disease.

With the exception of an enlarged and soft spleen, the pathological changes are limited to the muscles. Any or all may be attacked. It was early stated that the muscles of the eye, tongue, heart and diaphragm were exempt, but later investigation has shown that the eye muscles were implicated once (Strümpell's case); the tongue three times (Jacoby's, Strümpell's and Köster's cases); the heart apparently twice (Kell's and one of Oppenheim's cases), and the diaphragm five times (Strümpell's, Köster's, Senator's second case, Wagner's second case, and Janowsky's and Wyssokowicz's case). Batten (57) states that the masseters usually escape. They were implicated in Boeck's, Strümpell's and Unverricht's second case.

The skin covering the muscles is firm and hard on palpation and does not pit on pressure, though oedema is usually present, but may be slight, as in Jacoby's case. On section the subcutaneous tissues present a firm, tense oedema and are usually infiltrated with a yellowish serous fluid.

Macroscopically the muscles may exhibit extensive changes, as Unverricht has shown in an illustration of their gross appearance in his first case. The muscles are swollen, pale red, or pale yellow in color, or may reveal occasionally yellowish gray or diffuse reddish streaks. Hepp considers the muscles to resemble those of the dog. They are often strongly infiltrated with serum and quite moist. In consistency they vary, being hard and firm or soft and boggy. They may be quite friable.[3] They are without lustre and of a dull opaque appearance. Haemorrhages are occasionally seen in them. Microscopically the changes are those of a parenchymatous and interstitial

[3] In Hepp's case the left rectus muscle was found ruptured at the autopsy.

inflammation and may vary in extent, being either focal or diffuse. Again, the different muscle changes may be seen occurring in one and the same fiber. These are frequently separated from one another by the existing oedema or the presence of mononuclear and polymorphonuclear leucocytes. Small haemorrhages may also be seen between them. The fibers themselves are found in all stages of degeneration. They are swollen, coarsely or finely granular, hyaline, or waxy—occasionally fatty. The striae are normal, indistinct, or invisible. Longitudinal or cross cleavage of the fiber has been found, and vacuoles have been described in four instances. In many cases there is an increase in the number of muscle nuclei. Typical interstitial foci of small round cells are found in the perivascular connective tissue and, to a lesser extent, between the muscles. In the subacute and chronic cases the increase in connective tissue may be quite marked in both the perimysium externum and internum, and solitary muscle fibers in the process of degeneration may be seen surrounded on all sides by connective tissue. In five cases the blood-vessels were somewhat dilated and filled with blood.

Wagner has described new muscle formation as taking place in some of the fibers that had undergone waxy degeneration, while Senator has noted an abnormally large number of muscle spindles, two or three being found in each section. Senator, Pfeiffer and Lorenz consider the acute interstitial changes to be primary in this disease, and the degenerative muscle findings to occur chiefly in the second stage. They base this assertion on the reports of the different observers of the pathological processes here met with.

Illustrations of the microscopic changes are given by Wagner (in his second case), Jacoby, Strümpell, and Senator. For the detailed findings described by each writer see the table to be found at the end of this article.

In seven of the autopsies broncho-pneumonia was found as a terminal infection.

Symptoms.—The disease generally attacks persons in the prime of life and in the best of health. Fuckel's case, however, was noted in a

girl after an attack of measles,[4] and both of Wagner's cases had pulmonary tuberculosis, the second one having tuberculous ulcers of the intestines. Senator's second patient was a diabetic.

The onset is almost always gradual with the prodromal symptoms of malaise, weakness, anorexia, or headache. Vomiting was seen in two cases. These symptoms may be of several days' to three weeks' duration, or even longer, as in one case. Occasionally they are absent.

Pain.—Vague rheumatoid pains are next complained of, as well as a stiffness or rigidity in the extremities and back. These pains quickly take on a more definite character and become localized in the muscles. Different muscle groups are then successively attacked. Eventually the whole skeleton musculature may be implicated. Later in the disease the pains are more severe and prevent the patient from making the slightest movement.

Fever is soon noted. It is usually of moderate intensity, and remittent in character in the later stages of the disease. It rarely exceeds 104° F. Just before death it may rise several degrees.

Oedema.—With the fever oedema appears and may implicate the whole body and extremities, the latter presenting at times a most ungainly appearance. It is generally first seen on the face, especially about the eyelids. After the skin inflammation is noticed, it becomes more intense and may remain localized over the affected muscles or spread to surrounding parts. The wrists and ankle-joints are usually spared.

Dermatitis.—Dermatitis is an early symptom and varies greatly in character, being in different cases an erythema, a pseudo-erysipelas, an urticaria, a roseola, or an inflammation resembling erythema nodosum. It may spread continuously or remain limited to the parts where it is first observed. At times it occurs only later in the disease.

Profuse *perspiration* and an enlarged spleen usually accompany the other symptoms.

[4] It is interesting to note that Jessen has but recently reported a case of polymyositis following measles (loc. cit.) It is not typical enough, however, to be classed as one of dermatomyositis.

Nervous System.—No disturbances of sensation are, as a rule, met with, and the nerves are not tender on palpation. The knee-jerks and the electrical reactions are usually either normal or diminished. Lewy got a partial reaction of degeneration in his case.

Stomatitis and *angina* are at times seen, either early or late, in the disease. In Lewy's case and in four of Oppenheim's ulceration of the mucous membranes was observed. On this account the latter has coined the word dermato-mucoso-myositis to more rightly name this disease.

The *urine* is usually normal but may contain albumin.

The course may be characterized by improvement and relapses in the subacute and chronic cases, and atrophy of the muscles may also be here observed. In four cases pigmentation of the skin was found after the dermatitis had disappeared.[5]

Complications.—As stated above, pulmonary tuberculosis, tuberculous ulcers of the intestines, and diabetes are complications that have been met with. Acute haemorrhagic nephritis was observed in Senator's second case, while Jacoby's patient had acute cirrhosis of the liver. In seven instances broncho-pneumonia was found as a terminal infection.

Prognosis.—As all the muscles in the body may be implicated, including those of respiration and deglutition, death may result from suffocation or broncho-pneumonia. Out of the total number of cases (twenty-eight in all) seventeen terminated fatally, so that the prognosis must always be grave. The outcome was fatal for the two patients in the two extremes of life.

Diagnosis.—As a rule no difficulty will be experienced in typical cases. Diseases presenting somewhat similar symptoms are: 1. Trichinosis; 2. Neuromyositis; 3. Infective myositis; and 4. Syphilitic myositis. In the first the initial gastro-intestinal disturbances and the discovery of trichinae in the stools and excised muscle; in 2 the chain of nervous phenomena and the absence of a dermatitis; in 3

[5] For detailed account of the special symptoms, one is referred to Lorenz's excellent and exhaustive article or to Oppenheim's last paper.

the presence of a focus of infection with the bacteriological report on the muscle examination; and in 4 the history of the patient and the objective findings will generally sufficiently differentiate the affection. It is also well to bear in mind that one of Oppenheim's cases turned out, four years later, to be a typical example of scleroderma.

Treatment.—This should be chiefly directed to the relief of pain and to the keeping up of the patient's nutrition. Various analgesiacs have been tried for the former object but no particular drug employed has as yet been found to offer any especial advantage over the others. There is no specific treatment.

From the general observations upon dermatomyositis we now proceed to the detailed consideration of our case.

Case.—John E., aet. 31 years (Medical History No. 9632), laborer, a dark complexioned negro, was admitted to the Johns Hopkins Hospital March 7, 1899, complaining of soreness in his legs, chest, and hands, swelling of his muscles and inability to use them.

Family History.—Unimportant.

Previous History.—The patient had measles and whooping-cough as a child. He also gave a somewhat vague history of malaria four years prior to admission to the hospital. He had had gonorrhoea nine or ten years ago. The attack lasted about seven days; there were no complications. He denied syphilis. He used tobacco sparingly and was a moderate beer drinker.

Work.—Had been working almost constantly since a boy in the stone quarries of Baltimore. His work had been that of a "trencher" and had obliged him to be exposed to all kinds of weather. He had often been drenched through by the snow or rain.

Food.—He had always had a special fondness for beef and pork. Had also eaten a good deal of sausage, bologna and especially blood-pudding. He would often make a meal off sausage which he would buy uncooked to carry with him to work and eat in that condition for his noon-day meal.

Present Illness.—About six weeks before admission he was obliged to stop work because his limbs were sore and swollen. He also suf-

fered from pain in his chest muscles. The onset of his disease began sometime before this but he did not know the exact date. He first noticed that the sole of his right foot pained him, especially on walking. It seemed also somewhat swollen. From here the pain and swelling travelled up to the calf of that leg, then into the thigh, and finally to the hip. Soon after this—about one week, he thinks—his left leg was similarly affected. From here the pain and swelling went to his chest.

The patient stayed at home for about four weeks, either in bed or sitting up in an easy chair. At the end of this time he went to work again but found he could do nothing, for his arms were very sore and weak. Both arms were then affected like the chest and lower extremities. The pain and swelling began in the right hand and went to the forearm, and arm, successively. The left arm was next similarly affected. He described the pains as a "burning like the toothache," and said they were mostly present when he moved.

For about a year he had been somewhat troubled with shortness of breath. He had some nausea and several attacks of vomiting during the present illness but none at the onset.

Recently he had been voiding large quantities of urine, sometimes being obliged to get up as many as six times at night to urinate. There was no ardor urinæ, but often a sharp pain during micturition and at times a sudden stoppage of the flow.

He had not had any diarrhoea or abdominal pain.

On admission the temperature was 100.2° F., respirations 18, pulse 52.

Physical Examination.—Patient was a stout, well developed man and lay in bed in dorsal decubitus. His expression was dull and listless. Lips and mucous membranes were of good color; the conjunctivae were muddy, but not jaundiced. Pupils were equal and reacted to light and accommodation. The eye movements were normal and no puffiness was noted about the lids. There was no oedema of the face or body and no implication of the muscles of mastication. No appearance of a dermatitis and no signs of a former luetic erup-

tion. No muscle atrophy detected. No swelling of the nerves. No sensitiveness to pressure of the nerve trunks, and no disturbances of sensation.

Thorax.—The pectoral muscles stood out rather prominently and seemed swollen. Some pain was complained of on palpation. The intercostals were apparently not implicated.

The lungs and heart were practically negative on examination. The pulse was 56 to the minute, regular in force and rhythm, and of good volume and tension. There was slight thickening of the vessel wall.

Abdomen negative. Spleen not palpable.

Extremities.—No oedema noted. Flexion, extension, adduction and abduction slight on account of the pain caused. The calf muscles were held very much contracted, and seemed to be quite swollen. The patient complained of much pain and tenderness in them as well as in the muscles of the thighs, forearms, and arms. In these latter regions the contractures and swellings were not as prominent, except in the triceps. Palpation was unsatisfactory on account of the pain elicited and consequently a rigid examination could not be made. The muscles apparently were hard and firm. When the patient lay quiet and motionless in his bed but slight pain was complained of. Marked lineæ atrophicae were seen on the flexor surfaces of both arms, especially about the elbow-joints. The grasps of both hands were feeble and the act was attended with pain. The dynamometer did not register in the right hand and only just registered in the left. The knee-jerks were slightly increased. When the patient endeavored to sit up in bed he held his back and legs very rigid. Great difficulty was experienced when he attempted to button his clothes. His gait was extremely unsteady and walking seemed very painful to him.

There was slight general glandular enlargement.

Blood Examination.—Red blood-corpuscles, 5,788,000; white blood-corpuscles, 5250; haemoglobin, 70 per cent.

The differential count was as follows:

Small mononuclears	18.00
Large mononuclears	9.75
Transitionals	1.25
Polymorphonuclears	64.25
Eosinophiles	6.75

The leucocytes were counted daily for about three weeks and averaged about 8000, the highest count being 12,800 on March 12. The red blood-corpuscles remained about the same as at the first examination but the haemoglobin decreased somewhat in percentage. Differential blood-counts were made from time to time and showed but little change. The last count, made two days before the patient's discharge, was as follows:

Small mononuclears	19.00
Transitionals + Large mononuclears	5.00
Polymorphonuclears	69.00
Eosinophiles	7.00

Shortly after his admission, as the suspicion of his having trichinosis was entertained, a small piece of his left gastrocnemius muscle was excised under cocaine anaesthesia. The examination of this teased in salt solution was negative for trichinae. The individual muscle fibers showed marked degeneration. They were studded with minute granules which varied slightly in size and looked like fatty granules. Many of the fibers had lost their transverse striation. There appeared to be a distinct increase in the number of muscle nuclei. The connective tissue was increased in amount and in places showed well-marked cellular infiltration. The further examination of this muscle will be elsewhere described.

After the patient had been in the hospital about two weeks the pain and muscle tenderness subsided, so that he could be up and about the ward. His gait was, however, extremely unsteady and walking was slightly painful. It was only accomplished when he placed his legs

far apart and frequently he had to use a chair to guide himself with. No Romberg symptom was at any time observed.

On March 18 three small nodules were noted on his hands. They were about the size of a split pea and looked somewhat like acne pustules. They were situated: (1) At the junction of the first and second phalanges of the left thumb; (2) in the mid-metacarpal region of the right thumb; and (3) in the mid-metacarpal region of the right little finger. Pain was complained of in them, especially at night. To the touch they were quite hard and firm. One was excised for microscopic examination as will be later mentioned.

The electrical examination made by Dr. Thomas " was difficult on account of the pain which contractions of the muscles caused. A good contraction could be brought out with moderately strong faradic currents applied to the nerves and by direct stimulation of the muscles. When the muscle was stimulated by the faradic current it remained in a continuous tetanic contraction for several seconds. This was not observed when a moderately strong galvanic current was used. When either pole of the galvanic current was placed over the nerve (the ulnar at elbow) and the current gradually increased, pain was complained of and the muscles were thrown into tetanic contraction. This contraction was not confined to the muscles supplied by the nerve but seemed to be due to voluntary effort on account of the pain. In this case it was most marked in the triceps."

On April 8 he complained of a " feeling as if something was crawling down from the elbows of each arm to the fingers." [6] This was especially noted at night. After about eleven days this symptom disappeared and the patient seemed to have better use of the muscles of his arms. The grasp in both hands then, though stronger, was still quite feeble. The pain in the muscles had entirely gone.

The electrical examination, made a few days later, showed no change except that the muscles were no longer thrown into a tetanic contraction by the faradic current. About this time Dr. Osler noticed a distinct myotonic contraction when the patient grasped a round

[6] Noted also in the cases of Hepp and Lewy, as well as in Wagner's second case.

object. The object was grasped but could not be dropped and his fingers fully extended. This was more observable in the right than in the left hand.

From then on till his discharge, on May 2, there was nothing special to record save that his strength was returning rapidly.

The urine on admission was pale amber in color, clear, 1023 in specific gravity, acid in reaction, negative for sugar but contained albumin to the amount of 0.15 per cent. There was no apparent sediment. Microscopically a few hyaline and granular casts were found. The urine gradually cleared up and at the last examination, made just before the patient's discharge, there was only a trace of albumin and no casts were seen.

Temperature on admission was 100.2°. It was normal the next day and so continued till March 12, when, without any apparent reason, it rose to 101.8°. It was normal again after three days and remained so till the patient's discharge (May 2, 1899).

His subsequent movements were lost track of for about a year, for, although he promised to come daily to the dispensary for electrical treatment, after one visit he disappeared. He was not known at the address he gave us.

He was admitted again to the Johns Hopkins Hospital on February 10, 1900, complaining of cough, soreness in his arms and weakness (Medical History No. 10,905).

Present Illness.—After his discharge from the hospital he had remained quite well and was working in a stone quarry, hammering stone, until nine weeks previous. The present attack occurred, he thought, because he got overheated on a warm day. Dizziness with impairment of vision were the first symptoms. These symptoms came on suddenly and obliged him to stop work. Since then he had remained at home and had complained of soreness and weakness of the trunk and extremities, especially of the right arm and of the left arm at the shoulder. He attributed the trouble in the arms to the fact that he used these muscles most and believed the muscles most used became sorest and weakest. Swelling of the muscles accompanied

this soreness and weakness. In addition to the above described symptoms he had suffered considerably from muscular cramps[1]—usually in his right arm. The forearm would be flexed on the arm during these attacks and the fingers partly bent and held close to each other laterally. The cramps generally lasted from ten to fifteen minutes but were sometimes of longer duration. He did not know how frequently they occurred, but they were never felt in both legs or both arms simultaneously. Pain, not of the cramp-like character, was also complained of in the muscles, especially of the right arm. It was worse on moving about, but was sometimes present when he was at rest.

Had had some attacks of vomiting, especially after eating meat or a good deal of food during his present illness. About ten days before entrance to the hospital he had a shaking chill.

Physical Examination.—The patient looked lethargic. Pain was complained of when the pectoral muscles were palpated. They felt very soft and flabby, even during strong contraction, as did the muscles elsewhere. Although the muscular development seemed enormous there was marked loss of muscular power. No oedema was noted; the spleen was not palpable. Myotonia was not present.

Measurements of Arms and Legs in Centimeters.

	Right.	Left.			Right.	Left
Forearm	31.5	31.5	Contracted	Forearm	32.5	32
Arm	34.5	34		Arm	39	37.5
Calf	36.5	34		Calf	39	38
Mid-thigh	57.5	57		Mid-thigh	61	59

Blood Examination.—Red blood-corpuscles, 6,600,000; white blood-corpuscles, 4300; haemoglobin, 80 per cent.

Differential blood-count:

Small mononuclears	32.7
Large mononuclears	7.0
Transitionals	2.0
Polymorphonuclears	53.3
Eosinophiles	5.0

[1] Hepp also has described this in the later stages of the disease.

Electrical Examination by Dr. Thomas showed that the facial nerve responded to a very slight strength of the faradic current on both sides. Direct stimulation of the muscle caused a tetanic contraction which persisted for several seconds and with stronger currents much pain was elicited. With the galvanic current an ordinary contraction was obtained if the current was weak, but a tetanic contraction if the current was of moderate strength. Direct stimulation of the platysma by the faradic current gave a tetanic contraction which lasted for several seconds after the stimulus was removed. The brachial plexus gave with the faradic a tetanic contraction of the muscles supplied by it. This persisted for several seconds after the current was broken. Direct stimulation (faradic) caused tetanus of the portion of the muscle stimulated and the contraction persisted. On stimulating with the galvanic current the right ulnar nerve at the elbow a sharp, quick contraction was obtained which subsided when the current was broken. Repeated, quick galvanic stimulation did not throw the muscles into tetanus, even with a fairly strong current. Direct stimulation of the right external popliteal nerve caused a contraction of the muscles, particularly of that portion under the electrode. This contraction persisted some time after the current was broken. With the galvanic current, stimulation of this nerve caused a sharp, quick contraction, which did not persist. Direct stimulation of the muscles caused a sharp, quick contraction.

Dr. Thomas also noted that when the patient grasped the hand of the observer but little force was used. When told to let go there was some delay. This was also noticed when he was told to grasp an object, squeeze it, and then let go.

On March 2, pain at the back of the neck was first complained of, and difficulty in turning his head was experienced. This soon vanished, however, and the soreness and weakness gradually subsided. The patient left the hospital in good condition March 21, 1900.

On admission the urine showed conditions similar to those found on his first entrance to the hospital. When he was discharged there was only a trace of albumin and no casts were demonstrated.

His temperature varied between normal and 99°. During his stay

a piece of muscle from the left biceps was excised for microscopic examination.

The patient this time also gave a wrong address and we have been unable to locate him.

Microscopic Examination of Excised Muscle.—Portions of the muscle first removed were hardened in alcohol, bichloride of mercury, and Zenker's fluid. Celloidin and paraffin were used as imbedding agents. The sections were mostly stained with haematoxylin, eosin, safranin, congo red, picrocarmine, and van Gieson's fluid. The Gram-Weigert stain failed to show the presence of bacteria in a number of sections examined. No animal parasites were found. In every section some muscle fibers presented a peculiar anomaly. As seen in cross section this consisted in a collection of fibrillae cut transversely and encircled by a band of fibrillae cut longitudinally (Plate XXX, Fig. 1). Instead of one collection of fibrillae three or more bundles of them were sometimes so surrounded (Plate XXXI, Fig. 3). The muscle fiber showing this anomaly could be normal in structure but more frequently signs of degeneration were found, affecting first the centrally placed, transversely cut fibrillae. These would stain more intensely with eosin, but still reveal the structures that go to make up the Cohnheim fields. In subsequent stages this was difficult or impossible to make out. Finally, a mass staining intensely with eosin was seen, presenting the characteristics of waxy degeneration (Plate XXXI, Fig. 3). The surrounding or encircling longitudinally cut bundles were rarely concerned (Plate XXXI, Fig. 3), but if implicated the degeneration would begin with the innermost fibrillae and pass thence out to the periphery. These fibers were never attacked until the whole transversely cut bundle had undergone waxy degeneration. Eventually the whole muscle anomaly could occasionally be seen to have suffered this change. In almost every instance the outer muscle fiber appeared contracted and shrunken away from its sarcolemma sheath.

In longitudinal sections similar changes were seen. The first stage in the degeneration seemed to consist in the contraction of the inner or now longitudinal fibrillae, by which the cross striations were ren-

dered more prominent (Plate XXX, Fig. 2). Then cleavage of these fibrillae would be noted and a subsequent waxy condition of them with a loss, eventually, of their cross striation. The whole anomaly would occasionally show a marked curling or twisting, such as is met with in degenerating muscle fibers (Plate XXX, Fig. 2).

The sarcolemma was generally normal in appearance but sometimes showed the peculiar blebbing or vascular condition (Plate XXXI, Fig. 4) which Hoen (58) has described in some degenerated voluntary muscle fibers of the uvula. This blebbing was somewhat rare and its origin and development could not be traced so well as in Hoen's case. The appearances seemed to correspond to his description except that the granular material in the blebs was only seen in a few instances and it could not be determined whether this came from the disintegration of the fibrillae. The nuclei occupied the center of the bleb, and their frequent absence could probably be accounted for by the plane of the section being cut above or below that containing the nucleus. The more advanced the degeneration the larger the bleb, but no completely destroyed fiber was observed.

The muscle nuclei presented, at times, a normal appearance, but frequently they were increased in number, swollen, vesicular in shape and contained one to two nucleoli with chromatin granules. They were distributed over the whole extent of the fiber and were often found between individual fibrillae of fibers that had undergone waxy degeneration. Where this degeneration was extreme the nuclei also showed changes. They would be of no definite shape, swollen or decreased in size, would stain intensely in haematoxylin, and would assume bizarre positions.

As a rule, these muscle anomalies were somewhat below the average size of the muscle fibers. They were round or oval in shape, and could be found singly, when they were generally larger in size, or in groups of six or more (Plate XXXI, Fig. 3). They were scattered over all the sections examined and seemed to bear no special relation to the muscle spindles or normal muscle fibers.

The muscle not taking part in this anomalous condition showed interesting changes. Many of the degenerations which have been

described in muscles could be seen. The fibers were normal in size, or swollen and oedematous, or atrophied. First parenchymatous changes were noted, as a result of which the striations failed to come out distinctly in staining. Then the different gradations of waxy degeneration could be made out, and, finally, a complete homogeneous mass, with no intimate muscle structure discernible, could be seen. This was especially marked at the cut ends of the fibers and was probably here due to surgical trauma, as Erb (59) and Weber (60) have pointed out. Occasionally, by the teasing of a fiber, a gap was observed, due to the retraction of the contractile substance, which showed waxy degeneration. The sarcolemma sheath enclosed this gap and within were found very fine granules which took the eosin stain. Owing to the lack of simultaneous retraction there could be seen, in places, irregular cross bands of muscle, also waxy in character. At times this waxy condition had entirely disappeared and the thickened sarcolemma sheath alone remained, filled with rather fine, eosin-staining granules (Plate XXXI, Fig. 6), or fibers presenting this change in only a few fibrillae could be seen, the remaining part exhibiting waxy degeneration (Plate XXXI, Fig. 6).

Longitudinal cleavage was observed here as in the fibers of the muscle anomaly (Plate XXXII, Fig. 7). Cross cleavage was also made out, but rather infrequently. It could not be determined whether the cleavage took place in the membrane of Krause or the plane of Hensen (Plate XXXII, Fig. 8).

In rare instances the bursting of a sarcolemma sheath and the spreading out of the contained fibrillae was noticed (Plate XXXII, Fig. 7). The sheath at the point of rupture always showed Hoen's vesicular degeneration, which had evidently weakened it and might account for its rupture during life. The phenomenon, however, might be a purely artificial one.

Fatty degeneration of the muscle was not observed in any of the sections examined.

Vacuolic degeneration was rare. In all the muscles so affected a swelling of the fiber was seen and, at times, a slight loss of the cross striation. The vacuoles were generally round or oval in shape, of

varying sizes, and so situated that their long axes were parallel to the long axes of the fibers. Their borders were sharp and well defined in every case and their contents consisted of a coarsely or finely granular material containing occasionally two or three nuclei (Plate XXXI, Fig. 5). At times the vacuoles occupied the periphery of the fiber, and then, as a rule, contained a nucleus. Vacuoles occasionally were present in such numbers that the fiber, on cross section, appeared riddled with them. As Schaefer (61) has described, their appearance seemed to be heralded by a round or oval, opaque, non-staining area in a muscle fiber.

In one place there was a considerable extravasation of blood between the fibers. The sarcolemma sheath showed the vesicular degeneration, as above described, rather infrequently. Occasionally the sheath was considerably thickened, especially if its fiber had been completely destroyed.

The nuclei in an unchanged fiber were normal, but with commencing degeneration they would greatly increase in numbers and occupy the center as well as the periphery of the fiber. By a still further increase in number they sometimes almost hid the structure of the muscle. They were oval or vesicular in shape and contained one to two nucleoli. Their long axes were, as a rule, parallel to those of the fiber. At times the nuclei would undergo degeneration, become shrivelled up, stain intensely and lie at right angles to the long axes of the fiber. They disappeared apparently by karyolysis. In no place where there was this proliferation of nuclei were new muscle fibers to be found, but occasionally distinctly reparative processes were observed. The nuclei would lengthen out and become like a rod (Plate XXXIII, Fig. 10). In these cases two or three nucleoli would be seen in them and indentations would indicate a commencing division. Finally, one of these rods would form fourteen or more nuclei (Plate XXXIII, Fig. 11). The next step was the taking on of protoplasm by these nuclei and their formation into myoblasts or sarcoblasts. They received this protoplasm at the expense of the contractile substance of the muscle fiber and consequently might be in hollows of the fiber or, exhausting the whole contractile

substance, might simply be enclosed by the sarcolemma sheath (Plate XXXIII, Fig. 12). Up to this point amitosis was alone observed, but now the myoblasts sometimes showed exquisite examples of karyokinesis (Plate XXXIII, Fig. 13). The new nuclei so formed did not appear to make new cells, but probably in a further stage would form the sarcolemma nuclei of a new muscle cell.

The formation of spindle-cells from these myoblasts was seen, but no new muscle fibers were made out. This might be due to the fact that the process had not continued long enough. Muscle regeneration by the longitudinal cleavage of the old fibers and the subsequent budding of the same was also not observed, though longitudinal cleavage of a collection of fibrillae from an old fiber was occasionally met with (Plate XXXII, Fig. 8).

The myoblastic cells, at times, showed degenerative processes. Their nuclei would then stain intensely and finally disappear, leaving the cell protoplasm which would then take on an intense eosin stain. By the total destruction of all these cells the sarcolemma sheath might be so pressed together by the surrounding fibers as to make it difficult of recognition.

Muscle spindles were, at times, noticed in the sections, but there was no increase in their frequency or any pathological change discernible.

Nerves.—The nerves seemed normal in structure, save that in some instances there was an increased number of nuclei in the sheath of Schwann.

Connective Tissue.—The increase in small round cells in the perimysium externum and internum was comparatively infrequent. It could be seen especially in the perivascular connective-tissue space of the former. Much more marked in these situations was the increase in connective tissue, which was more noticeable in the perimysium externum. The tissue was oedematous and very loose in structure. Occasionally it was seen taking the place of a completely degenerated muscle fiber, or a collection of fibers (Plate XXXII, Fig. 8). It was especially rich in newly formed blood-vessels, and where it bordered on a degenerated muscle fiber it could be seen sending some shoots

across the fiber, if longitudinally cut, and, by further development of other blood-vessels and loose connective tissue, the position of the fiber might be completely occupied by these structures. The increase in fat cells was, at times, quite marked (Plate XXXII, Fig. 9). Occasionally the connective tissue underwent a waxy or granular change and an eosin-staining, granular mass might be alone discernible. In some places large multinucleated giant cells [8] were noted in the connective tissue, near degenerated muscle fibers.

The small nodules, referred to in the patient's first history, on sectioning and staining, proved to be small subcutaneous abscesses. There was nothing specially remarkable about them. No bacteria were noted in them by the Gram-Weigert stain.

The muscle excised from the patient's left biceps on his second admission was hardened in alcohol, embedded in celloidin and stained with haematoxylin and eosin. I have been unable to see these sections as they were destroyed during a small-pox epidemic in the ward. Dr. Harris, who was in charge of the patient at that time, has written to me that the muscle anomaly was wanting in these sections, but a distinct myositis was present, which consisted, especially, in a great increase in connective tissue between the fibers.

It seems fair to conclude that the above case was one of dermatomyositis, although it is unfortunate that in the light of our further knowledge we have been unable to question the patient more minutely on some of the symptoms in this disease. The swelling spoken of as present at the onset was probably oedema, which is always associated with dermatomyositis. No oedema was noted on his admission to the hospital. He was not asked as to a skin eruption, but if it consisted of merely an erythema he might easily have overlooked it on account of his color. Further information as to fever and some other points is also lacking. I regret I did not pay any attention to the macroscopic condition of the portion of muscle first removed. Microscopically the findings [9] are identical with those seen in this affection.

I have been unable to find a satisfactory explanation for the muscle

[8] Seen also by Wagner in his second case.

[9] Frohmann describes peculiar findings in his case, but they are too indefinite for us to judge whether or no he observed this anomaly.

anomaly. It has been described in two instances and two explanations have been given for its occurrence. Both, however, seem unlikely and rather fanciful. Bataillon (62) found it in frog larva. He considers it a degenerative process and says it begins by the tearing of certain of the outermost fibrillae of a muscle fiber. These torn fibrillae then circumscribe those centrally located, and are more or less obliquely placed. They run in all directions and are plainly separated from the central bundle. Often they show a more regular arrangement and by cross section may be seen to encircle, as a ring, with longitudinal fibrillae, the transversely cut fiber. The regularity of their cross and radial striations, as well as their staining properties, speaks strongly against this view. J. Schaffer (63), on the other hand, thinks that the muscle is not at all concerned, but that the encircling ring or band belongs to the sarcolemma, which, by a peculiar arrangement, simulates muscle striations. It is generally found in atrophied fibers of which the sarcolemma sheath has ruptured. After the rupture the sheath is retracted and drawn down in folds over the muscle fiber, below the site of rupture. The fiber above this retraction is without its sheath and the fibrillae are here widely spread apart. The successive infoldings of the retracted sarcolemma sheath causes the appearance of the circular striations. Our case, in Figs. 1 and 3, seems to refute this theory, as the ring or band can be plainly seen shrunken away from the surrounding sarcolemma sheath. (Plate XXX, Fig. 2, appears to show that this ring or band is muscle, cut in cross section.) The radial striations may possibly be due, Schaffer thinks, to the effect of the acids used in Flemming's solution—the hardening agent employed. Some of the tissue was hardened in Müller's fluid and similar pictures were seen, due, as Schaffer conjectures, to a contraction caused by the embedding process. The muscle in this case was from the gastrocnemius of a man. The description and plates correspond quite closely to the findings in our case, but some minor differences exist. In the lower types of vertebrates and invertebrates a bundle of transversely cut fibrillae is found, in cross sections of muscle, surrounded by a band of longitudinal fibrillae, which are in turn encircled by sarco-

lemma with a layer of undifferentiated sarcoplasm. Kölliker (64), Rollet (65), and others have described such pictures. Our anomaly, consequently, may be looked upon as a structure reverting to that seen in the lower types. Atavism, however, does not explain, but merely places the question in another phase.

In the other instance found in man it was also seen in the gastrocnemius muscle, so it may be a normal structure of that muscle and have some hitherto unknown function. It is our purpose to study further the histological structure of gastrocnemius muscles to see how frequently this so-called anomaly occurs and if it has any function of its own. It is interesting to note that it was not found in Kell's or one of Oppenheim's cases of dermatomyositis, though the gastrocnemius muscle was examined.

It is not inconceivable that what I have called above a muscle anomaly may be merely the result of pathological changes in the muscles. The blebbing of the sarcolemma, if of pathological occurrence, would doubtless weaken the muscle sheath and possibly a rupture might subsequently ensue. The outermost muscle fibrillae of the fiber, then no longer closely confined, would have a tendency to spread out. If consequently a cross section of this fiber were cut at a point where these fibrillae have begun to spread out a picture similar to the so-called anomaly (see Plate XXX, Fig. 1) would be obtained.

I have, also, been unable to explain why degeneration begins at the center of the anomaly and goes outward towards the periphery. In cross sections the longitudinal band seemed to press the transverse fibrillae very closely, and by this constriction the nutrition of the latter might be interfered with and the final result be degeneration of the centrally placed muscle bundle. I merely offer this as a possible partial explanation.

I am indebted to many friends who have looked over my sections and given helpful suggestions; especially do I desire to thank Dr. Welch for his kindly aid and valuable advice, and Dr. Osler for allowing me to report this case.

I am under obligations to Dr. Wolfe, of Hartford, Conn., for the photomicrographs which accompany this article.

Reporter.	Year.	Sex.	Age.	Occupation.	Onset.	Fever.	Parts Implicated.
1. E. Wagner.	1863	Woman.	43	Servant.	Swelling of both arms.	Yes.	Neck, thorax, abdomen and arms.
2. Potain.	1875	Man.	17	Farmhand.	Malaise, weakness, pains in extremities, and redness of eye-lids.	Yes.	Pharynx, arms and legs.
3. E. Wagner.	1887	Woman.	34	Cook.	Pain in back and sacrum.	Yes.	Pharynx, neck, thorax, diaphragm, arms and legs.
4. Unverricht and Marchand.	1887	Man.	24	Stone-mason.	Dragging pains in arms and legs.	Yes.	Pharynx, neck, thorax, b'k, abdomen, arms and legs.
5. Hepp and Jackson.	1887	Woman.	36	.....	Malaise, weakness and depression.	Yes.	Pharynx, larynx, neck, thorax, back, abd'n, arms and legs.
6. Jacoby.	1888	Man.	38	Machinist.	Feeling of tension in right calf.	Yes.	Tongue, thorax, back, abdomen, arms and legs.
7. Löwenfeld.	1890	Man.	50	Postman.	Pain in left calf.	Yes.	Pharynx, neck, abdomen, arms and legs.
8. Unverricht.	1891	Woman.	39	....	Swelling of both legs and urticaria.	Yes.	Face, neck, thorax, back, abdomen, arms and legs.
9. Strümpell.	1891	Man.	70	Gardener.	Nausea, vomiting, headache, and general malaise.	Yes.	Face, eyes, tongue, pharynx, diaphragm, arms and legs.
10. Senator.	1891	Man.	50	Baker.	Pain in right leg.	Yes.	Thorax, arms and legs.
11. Senator.	1891	Man.	40	Restaurant-keeper.	Weakness, anorexia, sleeplessness, dragging pain in back and painful stiffness in limbs.	Yes.	Larynx, diaphragm, abdomen, arms and legs.
12. Boeck.	1892	Man.	21	Laborer.	Violent pains in loins and thighs.	Yes.	Face, neck, thorax, abdomen, arms and legs.
13. Fuckel.	1892	Girl.	8	.....	Diarrhœa and slight fever.	Yes.	Arms and legs.
14. Lewy.	1893	Woman.	25	.....	Malaise, cough and anorexia.	Yes.	Thorax, abdomen, arms and legs.
15. Köster.	1895	Woman.	36	.....	Malaise and weakness.	No.	Face, tongue, pharynx, larynx, neck, thorax, back, abd'n, diaphr'm, arms and legs.
16. Kell.	1896	Man.	23	Sailor.	Pain in right calf.	Yes.	Pharynx, larynx, thorax, arms and legs.
17. Frohmann.	1899	Man.	42	Inspector.	Malaise and pain in thighs.	?	Arms and legs.
18. Lepine and Bonnet.	1901	Man.	59	Carpenter.	Intermittent, dull pains in the head.	No.	Face, back and arms.
19. Janowsky and Wyssokowicz.	1901	Woman.	23	Servant.	Pain in arms.	Yes.	Pharynx, larynx, neck, diaphragm, abd'n, arms, legs and sphincter ani muscle.
20. Bacialli.	1902	Man.	39	Clerk.	Vague rheumatoid pains in upper part of body, especially the arms.	Yes.	Pharynx, neck, thorax, arms and legs.
21. Oppenheim.	1903	Man.	38	.....	Malaise, anorexia, chilly sensations.	Yes.	Pharynx, arms and legs.
22. Oppenheim.	1903	Woman.	40	.....	Erythema, fever, pain in neck and arms.	Yes.	Pharynx, neck, arms and legs.
23. Oppenheim.	1903	Woman.	28	.....	Malaise, fever, profuse perspiration.	Yes.	Pharynx, tongue, arms and legs.
24. Oppenheim.	1903	Man.	48	Merchant.	Fever, roseola, pain in extremities.	Yes.	Pharynx, arms and legs.
25. Oppenheim.	1903	Man.	60	Gymnasium instructor.	Angina, pain in extremities, fever.	Yes.	Pharynx, larynx, arms and legs.
26. Christen.	1903	Boy.	10	Student.	Pemphigus-like eruption, vague rheumatoid pains.	Yes.	Face, abdomen, arms and legs.
27. Forcheimer.	1903	Woman.	40	.....	Irritation of skin and mucous membrane, headache, pain in various parts of body.	....	Thorax and legs.
28. Steiner.	1904	Man.	31	Stone-quarryman.	Pain and swelling in right foot.	Yes.	Thorax, arms and legs.

CASES.

Skin Changes.	Atrophy.	Duration.	Result.	Complications.	Cause of Death.	Remarks.
Erythema.	No.	19 days.	Death.	Pulmonary tuberculosis.	....	Autopsy.
Erythema.	No.	6 mos.	Death.	Broncho-pneumonia.	Broncho-pneumonia.	Autopsy.
Erysipelatous eruption.	No.	2 mos.	Death.	Pulmonary tuberculosis.	Broncho-pneumonia.	Autopsy by Huber.
Urticaria.	No.	5 wks.	Death.	Broncho-pneumonia.	Broncho-pneumonia.	Autopsy by Marchand.
Roseola.	No.	11 wks.	Death.	Broncho-pneumonia.	Broncho-pneumonia.	Autopsy by von Recklinghausen.
Erysipelatous eruption.	Yes.	2½ yrs.	Death.	Acute cirrhosis of liver. Bronc'p'a.	Broncho-pneumonia.	No autopsy. Muscle excised during life.
Roseola. Skin pigmentation.	Yes.	16 mos.	Death.	Bronchitis. Myocarditis.	... .	No autopsy.
Urticaria.	Yes.	6 mos.	Recovery.	.....	.....	....
Erysipelatous eruption.	No.	6 wks.	Death.	Bronchitis.	.....	Autopsy by Hauser.
Eruption like erythema nodosum.	No.	15 days.	Death.	Pneumonia. Diabetes.	Pneumonia.	No autopsy. Muscle excised at death.
Erysipelatous eruption.	No.	2 mos.	Recovery.	Bronchitis. Nephritis.	.....	.....
.....	No.	3 mos.	Recovery.	Gonorrhœa.	....	.....
Erythema. Urticaria.	No.	8 days.	Death.	Bronchitis.	.....	No autopsy. Muscle excised at death.
Roseola purpura.	Yes.	2½ mos.	Recovery.	Menorrhagia. Bronchitis.	.....	.....
Erythema, eczema.	No.	9 mos.	Death.	Broncho-pneumonia.	Broncho-pneumonia.	Autopsy.
Roseola, urticaria.	No.	7 days.	Death.	Bronchitis.	Suffocation.	Autopsy. Muscle excised during life.
Erythema.	No.	7 mos.	Death.	.....	.....	Muscle excised during life.
None.	No.	9 mos.	Recovery.	....	.....	.....
Erythema. Skin pigmentation.	No.	5 mos.	Death.	Broncho-pneumonia. Round duodenal ulcers.	Broncho-pneumonia.	Autopsy by Wyssokowicz.
Papular erythema.	No.	5 mos.	Death.	.....	Suffocation.	Autopsy.
Erythema. Skin pigmentation.	Yes.	8 mos.	Recovery.	.....	....	.....
Erythema.	Yes.	?	?	.....	.....	.....
Papular eruption.	Yes.	3 mos.	Death.	Epistaxis. Ulcer'n of mucous mem'e.	.....	No autopsy. Muscle excised during life.
Roseola. Desquamation.	Yes.	3 mos.	Recovery.	Ulceration of mucous membrane.	.....	.....
Erythema.	Yes.	4 mos.	Death.	Myocarditis. Ulc'n of mucous mem'e.	.....	No autopsy.
.....	No.	5 wks.	Recovery.	Epididymitis. Tuberc's of knee sev'l yr's prev's to at'k.	.....	.....
Pustular eruption, erythema, urticaria. Skin pigmentation. Desquamation.	Yes.	2½ mos.	Recovery.	....	.....	....
.....	No.	.....	Recovery.	.....	....	Muscle excised during life.

RÉSUMÉ OF PATHOLOGICAL FINDINGS.

E. WAGNER.—Muscles pale, greyish-red in color. On section the muscles were quite fragile and showed occasionally haemorrhages between the fibers.

Different grades of waxy degeneration to complete destruction of the contractile substance. An extreme grade of fatty metamorphosis. At times both of these changes are seen in the same fibrillae. No vacuoles, but an increase in muscle nuclei. Connective tissue unchanged. Numerous diffuse, very small foci of pus corpuscles. The nerves showed a waxy and fatty degeneration. Muscles of breast, abdomen, neck, upper arm and forearm examined and found implicated.

POTAIN.—Subcutaneous tissues infiltrated with serum.

Muscles pale and permeated with serum.

Many fibers exhibited granular degeneration. The striations were frequently indistinct. Spleen enlarged. Muscle from left forearm examined and found implicated.

E. WAGNER.—Subcutaneous tissues infiltrated with serum.

The muscles mostly of a uniform pale red color. In the flexors of the forearm spots and streaks of very pale appearance alternate with darker areas. The muscles are permeated with serum of a peculiar hard consistency, and are more fragile than normal.

Fibers swollen and showed parenchymatous or waxy degeneration in different stages, as well as fatty changes. The striae faint or invisible. Atrophy of single fibers with simultaneous nuclear increase, or vacuoles without nuclear increase or atrophy. Two or more pathological processes mostly found near each other. Diffuse and circumscribed serous infiltration between the muscles and in the connective tissue. Connective tissue showed proliferated nuclei and single multinucleated giant cells. Regenerative processes of the muscle and connective tissue seen. In places dilatation of capillaries and small haemorrhagic extravasations. Pectoralis major, intercostals, rectus abdominus, diaphragm, triceps brachii, iliacus, gluteus major and soleus examined and found implicated.

UNVERRICHT AND MARCHAND.—Firm, tense oedema of subcutaneous tissues, which are permeated by a yellowish serous fluid.

The muscles are generally of a dark reddish-brown color. They are soft, fragile and without lustre. On cross section pale, slightly transparent, light grey streaks alternate with dark red areas. When one predominates it gives its color to the muscles.

Fibers swollen and showed different stages of a hyaline or waxy degeneration and a finely granular cloudiness. Occasionally fatty degeneration was met with. Longitudinal and cross cleavage. Striae in places completely gone. No vacuoles and no increase in muscle nuclei. Interstitial tissue infiltrated with small round cells. Blood-vessels dilated and filled with blood. Occasionally large or small haemorrhagic extravasations noted. Spleen not enlarged. Extensors of arms (except triceps), extensors of thigh (except sartorious rectus and vasti) and extensors of toes much implicated. Sterno-

cleido-mastoid, pectoralis major and psoas muscles considerably changed. Flexors of thighs and legs as well as muscles of the back only slightly implicated. Abdominal muscles almost unchanged.

HEPP AND JACKSON.—Firm, tense oedema of subcutaneous tissues. Muscles pale and generally yellow in color, though occasional haemorrhagic areas are seen. They resemble the muscles of a dog and are markedly infiltrated with serum.

Different grades of hyaline or waxy degeneration to complete destruction of contractile substance. No fatty or granular degeneration observed. Striae faint or lacking. No vacuoles and no increase in muscle nuclei. Interstitial changes slight, but round-cell infiltration was found in the perivascular connective tissue or near degenerated fibers; in the latter places especially in the empty sarcolemma sheaths. Spleen enlarged. Rectus abdominis, triceps brachii, quadriceps femoris, pharynx and other muscles examined and found implicated.

JACOBY.—Coarsely granular and waxy degenerations in different stages. Fatty degeneration also noted. Longitudinal cleavage. Transverse striae generally lacking. Vacuoles and an increase in muscle nuclei. Increase in connective tissue, which is myxomatous or fibrous in structure. Perimysium externum, at times, exhibited fatty or waxy changes. Some nerves showed inflammatory changes, or an increase of nuclei in the sheath of Schwann. Left supinator longus and right gastrocnemius examined and found implicated.

STRÜMPELL.—Muscles paler than normal and yellower in color.

Fibers swollen or atrophied and showed a hyaline or waxy degeneration or a finely granular cloudiness. No fatty degeneration noted. Longitudinal and cross cleavage. Transverse striae generally absent. Vacuoles and an increase in muscle nuclei. Occasionally a new formation of connective tissue between the fibers. Typical interstitial foci of small round cells, especially in the perivascular connective tissue. Capillaries dilated and filled with blood. Spleen enlarged. Eye, tongue, pharynx, deltoid muscles of arms, lower extremities and diaphragm examined and found implicated.

SENATOR.—Subcutaneous tissues infiltrated with a sero-purulent fluid.

Muscles paler than normal.

No muscle degeneration. No vacuoles but an increase in muscle nuclei. Connective tissue increased and oedematous. Interstitial changes most marked. Infiltration of round cells (mononuclear and polymorphonuclear leucocytes) seen everywhere, especially in the perivascular connective tissue. Blood-vessels dilated and filled with blood. Small masses of extravasated blood occasionally noted. Great increase in muscle spindles. Right biceps brachii examined and found implicated.

FUCKEL.—Subcutaneous tissues infiltrated with serum.

Muscles somewhat swollen, darker than normal and haemorrhagic. They are quite fragile and lack their normal lustre.

Fibers changed in places to cloudy granules.

Transverse striations partly destroyed.

Muscle from extensors of upper arms examined and found implicated.

KÖSTER.—The muscles were pale, not tense, but quite firm.

Some fibers showed finely or coarsely granular, or hyaline degeneration. No fatty change observed. Longitudinal cleavage. Transverse and longitudinal striae singly or both wanting at times. No vacuoles and no increase in muscle nuclei. Interstitial foci of small round cells especially in the perivascular connective tissue. Capillaries and smaller blood-vessels dilated and filled with blood. Some free red blood-corpuscles in the muscles. Spleen not enlarged. Biceps, pectorals, intercostals, peroneus, tongue, diaphragm, larynx and pharynx muscles examined and found implicated. Pharynx and intercostal muscles most changed.

KELL.—Many fibers swollen and some showed granular and hyaline degeneration. Striae partly or wholly absent. No vacuoles. Between the fibers many large cells with large nuclei (proliferating cells [?]). In places periarteritis.

Spleen enlarged. Supinator longus, pronator, intercostals, heart and gastrocnemius examined and found implicated.

FROHMANN.—Granular degeneration of many fibers and loss of cross striations. Waxy degeneration. Increase in nuclei. Peculiar puckering and folding of the sarcolemma. Slight interstitial changes of an inflammatory nature.

JANOWSKY AND WYSSOKOWICZ.—The muscles are pale and of a yellowish-grey color. They are permeated with serum and of a peculiar hard consistency.

Fibers atrophied and showed a granular and waxy degeneration. Marked increase in connective tissue. There was only a slight cellular infiltration. The nerves showed no inflammatory change. Spleen not enlarged. Muscles of back, upper and lower extremities, throat, diaphragm and sphincter ani examined and found implicated.

BACIALLI.—Muscles paler than normal.

Fibers swollen or atrophied. They showed different stages of hyaline and waxy degeneration with complete destruction of contractile substance. Longitudinal cleavage. Vacuoles and an increase in muscle nuclei. Marked increase in interstitial connective tissue with abundant infiltration of white blood-corpuscles and here and there small haemorrhagic extravasations. Spleen enlarged.

OPPENHEIM.—Piece of muscle excised from the gastrocnemius showed the characteristic changes of a severe interstitial and parenchymatous myositis.

BIBLIOGRAPHY.

1. Lorenz.—Die Muskelerkrankungen. Wien, 1898, 120.

2. Wagner. E.—Ein Fall von acuter Polymyositis. *Deutsches Archiv f. klin. Med.,* Leipz., 1886-7, xl, 241.

3. Unverricht.—Ueber eine eigenthümliche Form von acuten Muskelentzündung mit einem der Trichinose ahnelnden des Krankheitsbilde. *München. med. Wchnschr.,* 1887, xxxiv, 488. Also, *Cor.-bl. d. allg. ärztl. Ver. v. Thüringen,* Weimar, 1887, xvi, 207. Polymyositis acuta progressiva. *Ztschr. f. klin. Med.,* 1887, xii, 533.

4. Hepp.—Ueber Pseudo-trichinose, eine besondere Form von acuter parenchymatöser Polymyositis. *Berl. klin. Wchnschr.,* 1887, xxiv, 297 and 322.

5. Marchand.—*Jahresbericht der schlesischen Gesellschaft f. vaterländ. Cultur,* 1880, lviii, 65. Also, *Breslauer ärztl. Ztschr.,* 1880, ii, 246.

6. Herrick.—Polymyositis acuta, with report of a case presumably of syphilitic origin. *Am. J. Med. Sc.,* 1896, cxi, 414.

7. Jacoby.—Subacute progressive Polymyositis. *J. Nerv. and Ment. Dis.,* 1888, xv, 697. Also, Ueber primäre subacute progressive Muskelentzündung. *Med. Monatschr.,* 1889, i, 10. And Schmidt's *Jahrb.,* 1889, ccxxi, 248.

8. Lewy.—Zur Lehre von der primären acuten Polymyositis. *Berl. klin. Wchnschr.,* 1893, xxx, 475.

9. Jackson.—Myositis universalis acuta infectiosa, with a case. *Boston Med. and Surg. J.,* 1887, cxvi, 498.

10. Wagner, E.—Fall einer seltenen Muskelkrankheit. *Archiv d. Heilk.,* 1863, iv, 282.

11. Potain.—Morve chronique de forme anormale. *Bull. de la Soc. méd des hôp. de Paris,* 1875, xii, 314. Also, *Union méd.,* Paris, 1876, xxi, 269.

12. Jacoby.—Op. cit.

13. Löwenfeld.—Ueber einen Fall von Polymyositis acuta. *München. med. Wchnschr.,* 1890, xxxvii, 531 and 549.

14. Unverricht.—Dermatomyositis acuta. *Deutsche med. Wchnschr.,* 1891, xvii, 41.

15. Boeck.—Et Tilfælde af Polymyositis acuta med Udgang i Helbredelse. *Norsk Mag. f. Lægevidensk.,* Christiania, 1891, 4 R., vii, 931. Also, *Centralbl. f. klin. Med.,* 1892, xiii, 405.

16. Strümpell.—Zur Kenntniss der primären acuten Polymyositis. *Deutsche Ztschr. f. Nervenh.,* 1891, i, 479.

17. Pfeiffer, R. — Die Polymyositis resp. Dermatomyositis acuta. *Centralbl. f. allg. Path. u. path. Anat.,* 1896, vii, 81.

18. Schnell.—Ueber Polymyositis. Dissertation, Würzburg, 1892.

19. Lewy.—Op. cit. and Ibid., 420 and 449.

20. Fraenkel.—Ueber eigenartig verlaufene septicopyämische Erkrankungen. *Deutsche med. Wchnschr.*, 1894, xx, 193, 227 and 245.

21. Buss.—Ein Fall von acuter Dermatomyositis. *Deutsche med. Wchnschr.*, 1894, xx, 788.

22. Herz.—Ueber gutartige Fälle von Dermatomyositis. *Deutsche med. Wchnschr.*, 1894, xx, 790.

23. Albu.—Ueber die Autointoxicationen des Intestinal tractus. Bonn, 1895, 97.

24. Schultze.—Ein Fall von Dermatomyositis chronica mit Ausgang in Muskelatrophie bei einem dreijährigen Knaben. *Deutsche Ztschr. f. Nervenh.*, 1895, vi, 245. Also, Ein Fall von Dermatomyositis chronica. Dissertation by George Müller, Bonn, 1894.

25. Schultzen.—Ueber Polymyositis, beziehungsweise Dermatomyositis acuta. Festschrift zur hundertjähr. Stiftungsfeier des med.-chir. Friedrich Wilhelm Institute, Berlin, 1895, 311.[10]

26. v. Kornilow.—Polymyositis primaria acuta. *Deutsche Ztschr. f. Nervenh.*, 1896, ix, 119.

27. Jollasse.—Ueber acute primäre Polymyositis. *Mitt. a. d. Hamb. Staatskrankenanst.*, 1897, i, 326.

28. Thiele.—Zur Aetiologie der Polymyositis acuta. *Wien. klin. Rundschau*, 1899, xxxiv, 573.

29. Neubauer.—Ein Fall von akuter Dermatomyositis. *Centralbl. f. innere Med.*, 1899, xii, 289.

30. Fuckel.—Ein Fall von Dermatomyositis acuta. *Cor.-bl. d. allg. ärztl. Ver. v. Thüringen*, 1892, 74.

31. Senator.—Ueber acute Polymyositis und Neuromyositis. *Deutsche med. Wchnschr.*, 1893, xix, 933.

32. Lewy.—Op. cit.

33. Köster.—Om primär Dermatomyositis acuta et chronica. *Nord. med. Ark.*, 1896, N. F. vi, No. xviii, 1. Also, *Centralbl. f. innere Med.*, 1897, xviii, 606.

34. Kell.—Report of three cases of polymyositis acuta; with report of post-mortem finding in one case. *J. Am. Med. Asso.*, 1896, xxvi, 967.

35. Gowers.—A lecture on Polymyositis. *Brit. Med. J.*, 1899, i, 65.

[10] I was unable to find this reference in the Surgeon General's Library. Lorenz speaks of the case as atypical as also does R. Pfeiffer, who gives a short resume of it. I have so classed it after reading what Pfeiffer says of it.

36. Frohmann. — Ueber primäre Polymyositis. *Deutsche med. Wchnschr.,* 1899, xxx, Vereins Beilage, No. 4, 24.

37. Bonnet.—Dermatomyosite. *Lyon Mèdical,* 1901, xxxiii, 10.

38. Lepine.—Polymyosite. *Revue de Mèd.,* 1901, xxi, 426.

39. Janowsky and Wyssokowicz.—Ein Fall von Dermatomyositis. *Deutsches Archiv f. klin. Med.,* 1901, lxxi, 491.

40. Bacialli.—Contribuzione allo studio della patogenesi e delle alterazione istologiche del tessuto musculare nella polimiosite acuta primaria. *Il. Policlinico,* 1902, ix, 16.

51. Oppenheim.—Ueber die Polymyositis. *Berl. klin. Wchnschr.,* 1903, xl, 381 and 416. Also, Zur Dermatomyositis. *Berl. klin. Wchnschr.,* 1899, xxxvi, 805.

42. Christen.—Ein Fall von Polymyositis acuta. *Cor.-bl. f. Schweiz. Aertzte,* 1903, xxiii, 822.

43. Forcheimer.—Dermato-myositis. *Boston Med. and Surg. J.,* 1903, cxlviii, 631.

44. Vincent.—Polymyosite infectieuse aiguë à récidives. *Bull. et mem. Soc. mèd. des hôp. de Paris,* 1903, 3 s., xx, 633.

45. Schüller.—Polymyositis im Kindesalter. *Jahrb. f. Kinderh.,* 1903, lviii, 193.

46. Jessen.—Polymyositis bei Masern. *Berl. klin. Wchnschr.,* 1904, xli, 847.

47. Lorenz.—Op. cit., and Ibid., 160.

48. Pfeiffer, R.—Op. cit.

49. Trinkler.—Kkazuist polymyositis acuta. *Chir. Vestnik.,* 1893, ix, 77.

50. Bauer.—Ein Fall von acuter hämorrhagischer Polymyositis. *Deutsches Archiv f. klin. Med.,* 1899, lxvi, 95.

51. Georgieoski.—A Case of Acute Haemorrhagic Polymyositis. *Bolnitsch. Gaz. Botkina,* 1900, xii, 696. See the reference in Thayer's article entitled, Notes on a Case of Acute Haemorrhagic Polymyositis. *Boston Med. and Surg. J.,* 1902, cxlvii, 313.

52. Pfeiffer, L.—Die Unverricht'sche Polymyositis gregarinosa oder Dermatomycosis acuta. Die Protozoën als Krankheitserreger, Jena, 1891, 2 Aufl., 208.

53. Virchow.—Giebt es eine Psorospermien-Krankheit bei Schweinen? Virchow's *Archiv,* 1866, xxxvii, 255.

54. Klebs.—Eine neue Form der primären Muskelatrophie (Atrophia muscularis parasitaria symmetrica). Festschrift Rudolf Virchow zu seinem 71. Geburtstage, Berlin, 1891, 31.

55. Köster.—Zur Kenntniss der Dermatomyositis. *Deutsche Ztschr. f. Nervenh.,* 1898, xii, 150.

56. Rosenblatt.—Ein seltener Fall von Erkrankung der kleinen Arterien der Muskeln und Nerven, der klinisch als Dermatomyositis imponirte. *Ztschr. f. klin. Med.,* 1897, xxiii, 547.

57. Batten.—Myositis. Allbutt's System of Medicine, 1899, vii, 461.

58. Hoen.—On a Form of Degeneration of Striated Muscle Met with in the Uvula. *The Journ. of Exp. Med.,* 1898, iii, 549.

59. Erb.—Bemerkungen über die sogenannte wachsartige Degeneration der quergestreiften Muskelfasern. Virchow's *Archiv,* 1868, xliii, 108.

60. Weber.—Ueber die Neubildung quergestreiften Muskelfasern, insbesondere die regenerative Neubildung derselben nach Verletzungen. Virchow's *Archiv,* 1867, xxxix, 216.

61. Schaefer.—Ueber die histologischen Veränderungen der quergestreiften Muskelgestreiften in der Peripherie von Geschwülsten. Virchow's *Archiv,* 1887, cx, 475.

62. Bataillon.—Recherches anatomiques et expérimentales sur la metamorphose des amphibiens anoures. *Ann. de l'universitè de Lyon,* 1891, ii, Fasc. 1.

63. Schaffer, J.—Beitrage zur Histologie und Histogenese der quergestreiften Muskelfasern des Menschen und einiger Wirbelthiere. *Sitzungsb. d. k. Akad. d. Wissensch., Math.-naturw. Cl.,* 1893, cii, 64.

64. Kölliker.—Handbuch der Gewebelehre des Menschen, Leipz., 1889, i, 358.

65. Rollet.—Ueber die Flossen muskeln des Seepferdchens (Hippocampus antiquorum) und über Muskelstructure im Allgemeinen. *Archiv f. Mikr. Anat.,* 1888, xxxii, 233.

DESCRIPTION OF PLATES.

All of the illustrations are photo-micrographs. The objectives and oculars were the Zeiss apochromatic; the condenser was Bauch and Lomb's, with a numerical aperture of 1; the illumination was the electric arc; Zetnow's light filter with methylene blue was used. The plates were Cramer's isochromatic (medium) and the exposure was two and a half seconds.

PLATE XXX.

Fig. 1. × 656. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

The muscle anomaly is here well shown in cross section, in the central part of the field. The innermost fibrillae are cut transversely and are surrounded by a longitudinal band of fibrillae, which is somewhat shrunken away from the sarcolemma. The cross striation of the longitudinal band is quite prominent. The anomaly is about equal in size to the other fibers.

Fig. 2. × 570 and reduced. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

The muscle anomaly represented cut longitudinally. The innermost fiber is contracted and somewhat curled on itself. Its striations are very prominent. To the right are waxy fibrillae, showing longitudinal cleavage and loss of some of the cross striations. The vesicular degeneration of the sarcolemma is to be noted in places.

Plate XXXI.

Fig. 3. × 570. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

Six of these muscle anomalies, about equal in size to the normal fibers, are seen. Some of the fibrillary bundles are stained deeply and have a homogeneous appearance. The larger one in the lower row shows this waxy change especially well and the innermost border of the longitudinal muscle layer is beginning to be similarly affected. The upper right anomaly shows three fibrillary bundles cut in cross section and surrounded by longitudinal fibrillae. In some the muscle is considerably shrunken away from the sarcolemma.

Fig 4. × 570 and reduced ½. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

The blebbing or vesicular degeneration of the sarcolemma. In a few blebs nuclei are to be seen. There is a great increase in nuclei in the muscle fiber on the left.

Fig. 5. × 570 and reduced ½. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

The swollen condition of a muscle fiber filled with vacuoles. The vacuoles are sharply defined, when well focused, and are generally fairly well filled with rather fine granules. A nucleus is to be seen in the innermost vacuole.

Fig. 6. × 570 and reduced ½. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

The swollen and degenerated sarcolemma sheath is here represented as quite well filled with rather fine granules. The fiber on the right is swollen and shows waxy degeneration together with this granular condition.

Plate XXXII.

Fig. 7. × 570 and reduced. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

The bursting of the sarcolemma sheath and the spreading out of the fibrillae. Above and below this spot the muscle fiber is contracted. An increase in muscle nuclei is seen. This bursting of the sarcolemma sheath may be due to its weakening caused by the vesicular degeneration at this place, or it may be an artificial phenomenon.

Fig. 8. × 570 and reduced. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

Increase of connective tissue at expense of the muscle which in places is broken up into bands or shows a waxy condition of the fibrillae. Cross and longitudinal cleavage is here seen. The connective tissue is loose and very oedematous. There is a marked increase in muscle nuclei.

Fig. 9. × 156 and reduced ½. Objective 16 mm. N. A. 30. Compensat. proj. ocular No. 6. Stain, van Gieson.

Increase of the perimysium externum. Many fat cells and new blood-vessels. In the upper part of the field a sarcolemma sheath containing myoblasts is to be noted. The muscle fibers show an increased number of nuclei.

PLATE XXXIII.

Fig. 10. × 570. Objective 6 mm. N. A. 95. Compensat. proj. ocular No 6. Stain, haematoxylin-eosin.

Rod-like nuclei in a row of four are to be seen in the center of a muscle fiber.

Fig. 11. × 570. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

By direct division of these rod-like nuclei long rows of nuclei can be found. In this figure a line of eleven can be seen.

Fig. 12. × 156. Objective 16 mm. N. A. 30. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

These muscle nuclei are here situated in the hollow of a muscle fiber and in an empty sarcolemma sheath and are surrounded by protoplasm. These cells are known as myoblasts or sarcoblasts.

Fig. 13. × 1390. Objective 2 mm. N. A. 1.42. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

One of the myoblasts seen in the last figure is shown under higher amplification undergoing karyokinesis.

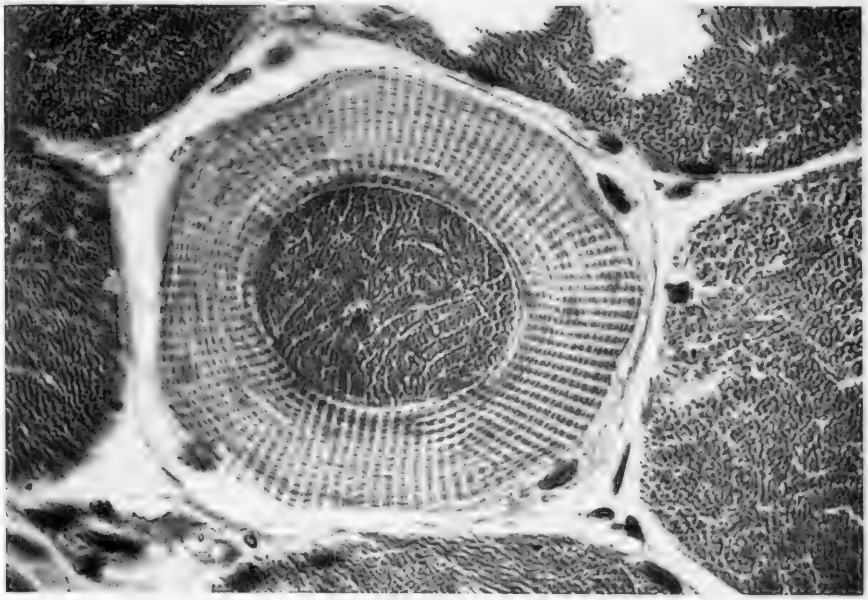

FIG. 1.

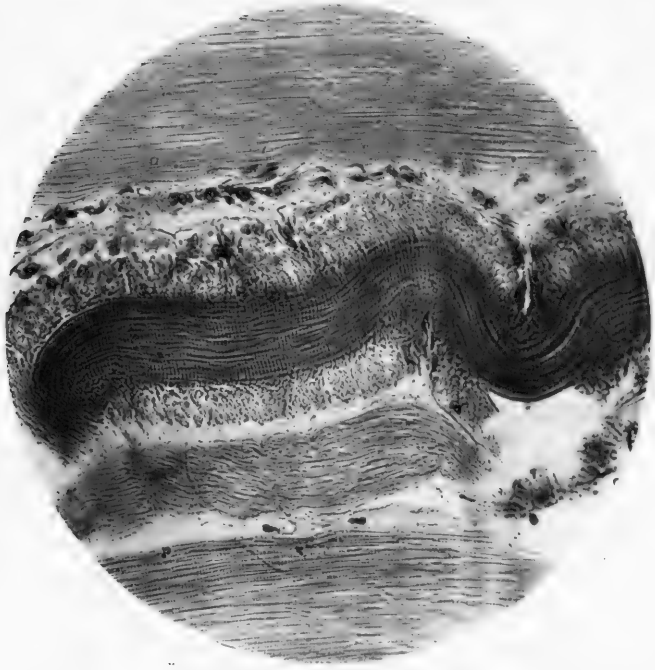

FIG. 2.

FIG. 3.

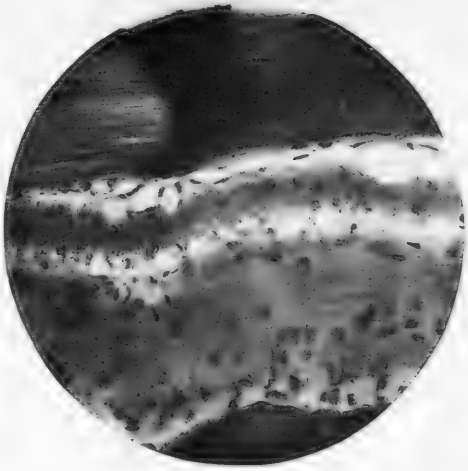

FIG. 4.

FIG. 5.

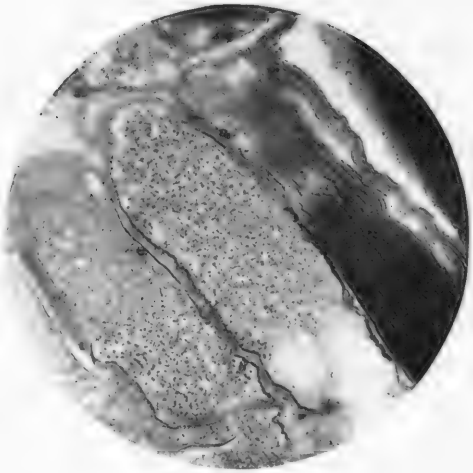

FIG. 6.

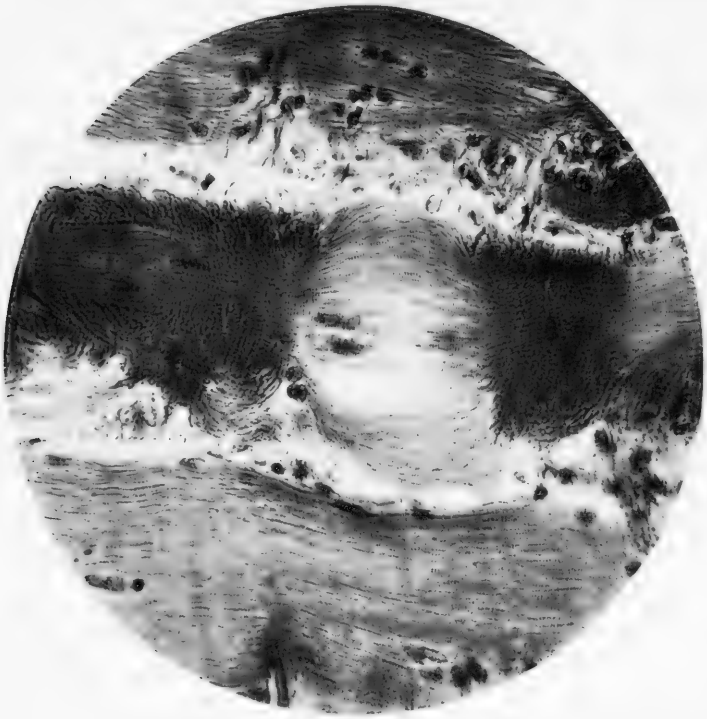

FIG. 7.

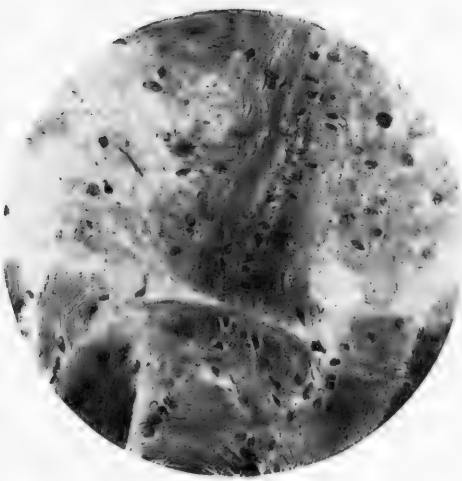

FIG. 8.

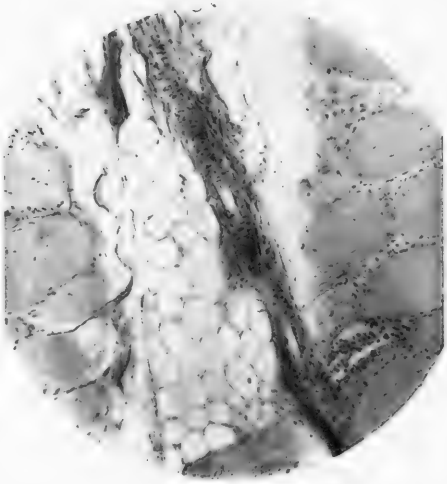

FIG. 9.

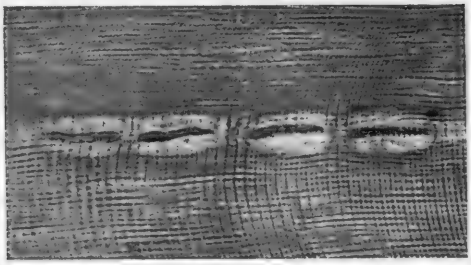

FIG. 10.

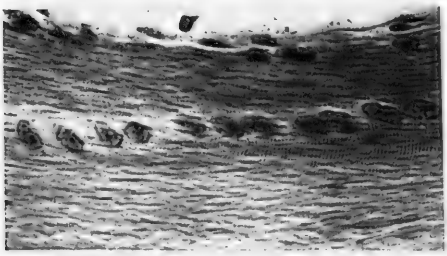

FIG. 11.

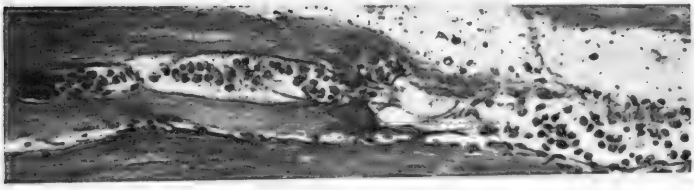

FIG. 12.

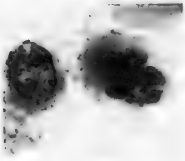

FIG. 13.

FURTHER OBSERVATIONS ON A PATHOGENIC MOULD FORMERLY DESCRIBED AS A PROTOZOON (COCCIDIOIDES IMMITIS, COCCIDIOIDES PYOGENES).

By W. OPHÜLS, M. D.

(From the Pathological Laboratory, Cooper Medical College, San Francisco.)

In June, 1900, Dr. H. C. Moffitt and myself published, in the *Philadelphia Medical Journal,* a preliminary report on "A New Pathogenic Mould," which formerly had been described as a protozoon, and had been named by Rixford and Gilchrist, *Coccidioides immitis* and *Coccidioides pyogenes.* It is the object of this paper to give a more detailed description of the observations communicated in the former article, and also to add some further developments which largely confirm the conclusions which we then drew from our first investigations.

I have also had the good fortune to find another case of this form of infection in a human being, a case that derives an additional interest from the fact that the localizations of the disease in it were in many ways different from those observed in former cases of this kind. Unfortunately the post-mortem examination in this last case was held very late after death and the usual routine bacteriological examination was, therefore, omitted. The unusual nature of the case was not apparent at autopsy and was recognized only when fresh material for animal experiments could no longer be obtained. The appearance of the parasites in the tissues is so typical, however, that a doubt whether this case belongs to the same category seems to be hardly justified.

Apart from a few early reports of coccidiosis in the human being (Hadden, Silcock, Bland-Sutton, all mentioned in *The Lancet,* 1889, II, and in the *Centralblatt für allgemeine Pathologie und pathologische Anatomie,* 1894), of which I have not been able to find descriptions accurate enough to decide whether they belong to our class of cases, there are, so far as I could make out, only four observations of

infections of this kind in the human being recorded in the literature. The first case was found by Posadas in Buenos Ayres, and briefly described by Wernicke in 1892, in the *Centralblatt für Bacteriologie und Parasitenkunde*. With his description of the case he gives some microphotographs which do not leave any doubt about the nature of the infection. Later, after the death of the patient, Posadas published a more elaborate paper on this same case in the *Revue de chirurgie* for March, 1900. The original unfortunately was not accessible to me and I had to be satisfied with a review in the *British Medical Journal* for April, 1900. The other three cases were observed in California, two of them by Dr. Rixford (later described by him in conjunction with Dr. Gilchrist in the *Johns Hopkins Hospital Reports,* I) and the third one by Dr. D. W. Montgomery (*British Journal of Derm.,* XII, 1900). In addition to these and the two cases described in this paper, I know of one more instance that was also seen by Dr. D. W. Montgomery * and examined by Dr. A. E. Taylor. Unfortunately the patient left San Francisco before I could avail myself of their invitation to examine him, and at the same time make some inoculation experiments. It seems, therefore, that the disease is not such a very rare one in California, and we can hope that new material will be forthcoming that will enable us to obtain more positive data about the one important point that now remains to be decided, namely, the source of spontaneous infection. It has remained doubtful whether the disease is transmitted from man to man, or whether the parasite lives somewhere in nature, or, what I suspect very strongly, whether the disease is originally an animal disease and only occasionally transmitted to man.

All former cases, with the exception of Montgomery's, seem to be examples of primary infection of the skin. Rixford and Gilchrist directly speak of a protozoic dermatitis. It is quite remarkable that both cases that I have seen should be different from this usual type. In both of them there were no primary cutaneous lesions

* This paper was written in 1901. Since then Drs. Montgomery, Ryffkogel and Morrow have published this case in the *Journal of Cutaneous Diseases*, January, 1905.

at all, and the infection had evidently started in the lungs and thence spread to other organs. In the first case,[1] the one studied by Dr. Moffitt and myself, the first clinical symptoms appeared in the chest, and at autopsy a very extensive involvement of the lungs was found. There was also an empyema on the left side in which the parasitic organisms and pseudo-diphtheria bacilli were present. From the lungs the disease had extended through adhesions into the diaphragm and further down to the retroperitoneal lymphglands. There had also occurred a generalization of the disease by way of the blood current leading to the formation of miliary abscesses in the spleen, submiliary tubercles in the liver, tubercles and chronic miliary abscesses in both kidneys, purulent periostitis and ostitis of the frontal bone and both tibias, with formation of abscesses and perforation through the skin, suppurative inflammation of both knee-joints, the right shoulder, both elbows and both wrists.

In the second case there were old lesions in the lungs. From the lungs the pericardium had become infected, an infection which resulted in an obliteration of the pericardial sac by fibrous adhesions and the formation of granulation tissue with submiliary tubercles and miliary abscesses in the pericardium at the basis of the heart. Either in the lungs or the pericardium—this point could not be definitely decided—the parasites must have gained access to the blood current, the infection of the blood resulting in this case in a basilar tubercular meningitis and a miliary tuberculosis of the spleen and both kidneys.

These observations prove the occurrence of a primary pulmonary infection in this disease very probably due to inhalation of the parasitic organism which causes it. As yet it is impossible to decide whether a primary intestinal infection by ingestion of the parasite is also possible.

In all six cases the pronounced tendency of the disease not only to produce very extensive lesions at the point of infection but also to spread by the way of the lymph- and blood-current is very marked.

[1] See detailed description of cases at the end of the paper.

All cases ended with the death of the patient. Both facts prove that the human being when once infected is eminently susceptible to the disease, and the scarcity of cases can be explained only by assuming that an occasion for infection is very rarely given.

MORPHOLOGY OF THE PARASITES IN THE TISSUES.

As far as the appearance of the parasites in the tissues is concerned, I have very little to add to the excellent description and very instructive plates given by Rixford and Gilchrist in their paper in the *Johns Hopkins Hospital Reports.*

The adult parasites are almost perfectly spherical organisms measuring about 30 μ in diameter. They consist of a central granular protoplasmic body and a peripheral double-contoured, highly refractile membrane. The thickness of the membrane varies a little, but in normal individuals it always has a considerable diameter—from about a fifth to a tenth of the radius of the whole parasite. Whether there is a definite nucleus has not as yet been made out. The protoplasm stains well with nuclear dyes and there are frequently larger, intensely staining spots in it. Vacuoles may be seen, but are not constant nor even very frequent.[2]

In old lesions one frequently encounters what seem to be degenerated forms. Either they show numerous small vacuoles, or the protoplasm shrinks, and the natural result is the falling together and folding in of the membrane. Such a form is represented in the lower half of Fig. 17. As I have found such forms not only in sections but also sometimes in fresh teased specimens, I do not believe that they can be regarded as artefacts, a possibility that is discussed by Rixford and Gilchrist.

Under favorable conditions the adult forms begin to sporulate. The first step is a cleavage of the protoplasm inside of the capsule into two parts.[3] These divide into four and so on, until a large number of spores have been formed, as is well described in Rixford and Gilchrist's paper and shown in their Figs. 8, 9, 10, 11 and 12

[2] In Rixford and Gilchrist's Case II they were almost constant.

[3] This earliest step, however, has never been actually observed.

in Plate XXVIII. The spores formed in this way are quite numerous, usually a hundred and more. Their protoplasm is homogeneous and highly refractile. The ultimate form of the spores seems to be largely the effect of their being crowded together very closely in the more or less unyielding capsule. The central ones are usually more or less S-like and sickel-shaped. The peripheral ones have the form of biconcave disks or biconcave plates of varying forms. One also finds pyramidal forms with more or less concave surfaces. (Examples of sporulating forms are found in Figs. 5, 16 and 23.)

Neither Rixford and Gilchrist nor I have ever been able to see any active motility in these spores. They sometimes stain very well with nuclear dyes, at other times very badly or not at all. I have the impression that the very last stages are the ones that stain so poorly. At least this much is sure, that after they have been discharged into the tissues through a tear in the capsule—a step that now follows—it is very difficult or impossible to recognize them, because they do not seem to take either nuclear or protoplasmic stains in a decided manner. In a few instances Rixford and Gilchrist have succeeded in staining these free spores with Gram's method.

On account of this difficulty of recognizing the spores after they have been discharged from the capsule, it is impossible to make any very positive statements as to how the young parasites develop from the spores. It is, however, likely that they originate from them by simple enlargement, in such a way that the protoplasm in the center of the spores enlarges and the spore membrane also grows and develops into the double-contoured membrane which envelops the adult parasite. At first there seems to be a considerable stretching of the membrane. At least the younger forms certainly have a thinner membrane than the older ones.

Sometimes, at least in my first case, the development of the young forms from the spores takes place within the old membrane. This seems to occur when the development of the parasite is an especially rapid one. The young forms certainly develop into the adult forms by simple enlargement. One can easily trace the different stages of development in the specimens.

In their Fig. 17 on Plate XXIX, and Fig. 50 on Plate XXXIII, Rixford and Gilchrist depict forms in which the outer surface of the membrane is full of small prickles. The former is a sporulating, the latter an adult form. They state that they observed these forms only in two or three instances out of many hundreds. I have never seen forms with prickles as long as those represented in the plates, which really would be better described as bristles. Short irregularities on the outside of the membrane and numerous short prickles in the same place, however, I have met with very frequently. They are best seen in fresh specimens and so far as my observations go are almost constant on the outside of the membranes of the sporulating forms.

Like Rixford and Gilchrist, I also have been unable to find any definite budding forms. The examples nearest to it, that I have ever seen, are represented in Fig. 29 and Fig. 30. In the latter it certainly looks more as if there was a formation of a small pedicle than that of a bud.

I have, however, quite frequently, especially in my animal experiments, seen two young parasites lying directly adjoining one another so that both together formed a figure 8. I call these, for the sake of brevity, figure-8 forms. In this case each of the two parasites is sometimes surrounded by a distinct membrane, as is shown in Rixford and Gilchrist's Fig. 34 on plate XXX, but at other times the two capsules are fused into one at the point where the parasites touch one another, so that forms are produced which in some cases might easily arouse the suspicion of a budding. However, the two parasites were always of the same size and there was never any direct continuity of the protoplasm.

Rixford and Gilchrist are undecided whether in their two cases they have to do with one and the same organism or whether the organisms in the two cases, although closely related, belong to different species. They say after the description of the second form:[4] "It is not even certain that the parasites in the two cases are not

[4] Rixford and Gilchrist, loc. cit. page 260.

identical species. Still the formation of the spores peripherally and the abundant production of pus with the second form of parasite are such striking peculiarities that for the present we propose the name Coccidioides pyogenes (in contradistinction to Coccidioides immitis) for this second variety of the parasite."

Both in my human cases and also in my animal experiments, which were all made with the parasites obtained from Case I, I have seen so many striking differences in the number and arrangement of the spores formed that I hardly think this point can be used as a means of differentiation between closely related species. As far as the abundant formation of pus in their Case II is concerned, I hope to be able to prove further on that this feature was due not to a difference in the parasites, but to the presence of large numbers of sporulating forms. I believe, therefore, that it will be better, until further evidence to the contrary is forthcoming, to regard the two cases of Rixford and Gilchrist as infections with the same organism.

Rixford and Gilchrist have already shown that with material containing the protozoon-like bodies just described it is possible to produce lesions in animals which, at least to a certain extent, resemble those which were observed in the human being. Subcutaneous inoculations only were attempted and the results of these were not at all constant. Of seven (two dogs and five rabbits) experiments only two (Dog 1 and Rabbit 5) proved successful. In the dog a chronic ulcer developed at the site of inoculation (leg) and an enlargement and abscess formation in the regional (inguinal) lymph-glands followed. In both lesions protozoon-like bodies were demonstrated. Both healed completely after excision. In the rabbit also a chronic ulcer developed which contained the protozoon-like bodies. The ulcer persisted for a long time (six months), but showed a tendency to decrease in size. A part of it was excised, after which the wound healed rapidly. There were no signs of a generalization of the infection.

The material used in these experiments, however, did not contain the parasite in pure culture.

Our experiment on Guinea-pig No. 1[5] shows that these animals also are not very susceptible to subcutaneous infection. When the guinea-pig was killed ten weeks after inoculation a very slight lesion was found at the point of inoculation and no involvement of the regionary lymph-glands nor any general infection.

That in man also the skin is not very susceptible to the infection Rixford and Gilchrist demonstrated by experimental inoculations of unaffected parts of the skin in their Case I. These inoculations in all instances proved unsuccessful. Our experiments on Guinea-pigs 2 and 3 prove, however, that the animal is very much more susceptible to intraperitoneal infection.

Further experiments with the protozoon-like bodies themselves on rabbits were not performed.

DEVELOPMENT OF THE PARASITE ON ARTIFICIAL MEDIA.

The first to obtain a development of the parasitic organisms on artificial media were Dr. H. C. Moffitt and Dr. May Ash. At the autopsy, which was performed by Dr. Moffitt, they inoculated slanting agar-agar tubes with material obtained from the kidney, lung, the abscess over the left eye, the left pleural cavity and spleen. In the cultures from the pleura and spleen a white mouldy growth developed which, when it was shown to me, I regarded as an accidental contamination, whereas Dr. Moffitt and Dr. Ash were quite ready to believe that it had something to do with the parasitic organism found in the tissues. When, however, Guinea-pig No. 2, which had received some of the pulmonary tissue of Case I injected into the peritoneal cavity, died showing lesions which in many respects resembled those of Case I, and which contained the typical parasites in pure culture, and when also cultures made at the autopsy from the pus near the testicles now developed the same mould in profusion and in pure culture, my attitude towards the mould naturally began to change.

It was at once recognized that in order to make sure of the true relation of the parasite found in the tissues and the mould in the

[5] A detailed record of the animal experiments is given at the end of the paper.

cultures to one another two things were necessary: first, if possible, to trace under the microscope the development of the mould from the protozoon-like bodies (the parasite in the tissues), and secondly, to attempt to reproduce lesions containing these protozoon-like bodies by inoculating animals with pure cultures of the mould. As we have already stated in our preliminary report, we succeeded in both respects.

On April 20, 1900, a little of the pus from an abscess near the testicle in Guinea-pig 3 was suspended in beef-tea in the form of a hanging drop. On examination the drop contained three protozoon-like forms. The slide was then incubated for twenty-four hours and next day a mycelium had developed from two of the three organisms that were present, very much in the same fashion as is represented in Figs. 32 and 33, which are drawn from later experiments. In order to exclude all possible error, this experiment was repeated a considerable number of times, always with the same result.

The experiments showed further that a development of the protozoon-like bodies into mycelia does not take place so long as the parasites are enclosed even in the smallest shreds of tissue. This explains why one usually obtains cultures only from abscesses, not from the more solid tubercle-like lesions.

In the second place, even when the parasites are free, they do not all grow out into mycelia. It seems that they must have at least reached the adult stage.

The development of the mycelia from the protozoon-like bodies occurs in the following manner: The thick membrane which surrounds the latter becomes very thin in one place and begins to evaginate over a bud from the enclosed protoplasm (see Fig. 32, upper left corner). These buds soon assume the form of coarse more or less cylindrical threads [6] which are either straight or somewhat wavy.

[6] In a case studied only very recently after completion of this paper, the first buds were more or less club-shaped, and later on several rows of club-shaped links developed around the protozoon-like body extending more or less in a radial direction away from it. The appearance was entirely that seen in certain saccharomyces that form hyphae in their cultures. More in the periphery of the growth, however, the hyphae became again cylindrical like those in Figs. 32 and 33.

At first the protoplasm forms one continuous mass in main body and buds, but after a while septa appear at various places in the buds and sometimes a partition also forms between the bud and the main body.

Fig. 34 represents what I believe to be the development of hyphae from a spore of the protozoon-like bodies. The specimen was made from an abscess that had developed at the base of the ear of Rabbit 4, after an injection of material from a pure culture of the mould into the subcutaneous tissue at this spot. The specimen, which was taken on the tenth day after the injection, was carefully examined and none of the spores of the mould, originally introduced with the pure culture, seemed to be left. After twenty-four hours in the incubator a budding of hyphae from small round bodies, like *a* in Fig. 34, was found in very many places. Since the pus contained many sporulating forms and also many empty shells, it must have contained many free endospores from the protozoon-like bodies, which, however, at the first examination were not specially noticed. I have stated above how difficult it is to recognize them after they have become scattered in the tissues. It is, therefore, very probable that these endospores enlarged (*a* in Fig. 34 is much larger than the endospores of the protozoon-like bodies) and then budded out into hyphae.

There is one more reason why these round bodies from which the hyphae developed could not be the originally injected spores from the mould, and that is found in their form. The difference is well seen in comparing Fig. 31, which represents a budding spore from a pure culture on artificial media, with Fig. 34.

In our preliminary report we state: "It also seems as if there occurred (in the hanging drop outside of the tissues) a limited development of the protozoon-like bodies when they are enclosed in shreds of tissue." This statement was based on some observations in which I had counted the number of parasites in shreds of tissue before and after incubation. It appeared then as if their number had slightly increased. Later counts, which perhaps were made more carefully, did not confirm the earlier ones, and I now believe that the discrepancy in the figures was due to the fact that after continued soaking in

beef-tea the smaller forms show better in the tissues, so that they could have been overlooked in the first count but were easily seen and registered in the second one.

The cultures obtained from Case I and from a number of the inoculated animals may be shortly described as follows:

Agar-agar (ordinary agar-agar, glycerin agar-agar, 2% glucose agar-agar). The growth develops slowly at room temperature, quite rapidly at 37° C. (thick membrane in 24 to 48 hours in incubator). On the surface of the medium a white mouldy growth is formed with a slightly wavy outline. The aërial hyphae are never very long, about .5 to 1 mm. Old pure cultures look as if they had been strewn over with just visible, bright, white particles of sand. From the lower surface of the growth numerous hyphae grow into the medium, by which the growth becomes very firmly attached to it. Only with a stout platinum needle is it possible to dig out portions of the growth from the surface. The agar-agar in the neighborhood of the growth soon shows a brown discoloration. The growth itself remains perfectly white, only very late when the medium dries out one observes a slight yellowish-brown discoloration. In the later stages of development, when the medium dries out, the growth retracts more in the middle than in the periphery. The membrane begins to show a slight irregular wrinkling but there is no marked folding.

Microscopic examination in the beginning of the development shows long, septate, branched hyphae (about 2 μ in diameter) which form an intricate meshwork. The hyphae within the medium always remain in this condition. In the aërial hyphae one observes later club-shaped swellings at the end and also the formation of spores. The spores originate at the end of the aërial hyphae within them. Three or four are formed at a time by a contraction of the protoplasm at regular distances with accompanying swellings at these places (Fig. 25). Later the contracted protoplasmic mass is shut off at both ends by a double-contoured membrane. The protoplasm that goes to form the spores is at first very granular, later highly refractile and homogeneous. The formed spores have biconvex sides, as is shown in Fig. 25. The places between the spores are entirely devoid of protoplasm and the membranous cylinder that connects the spores after they have been formed, seems to be very brittle, so that after a while the spores break off and form irregular clusters around the ends of the aërial hyphae. In these clusters the spores at first usually adhere to one another in rows, but later they break apart and become

single. At the ends of the isolated spores one can almost invariably see pieces of the empty intermediate link that existed between them.

From this description one will see that the spores are typical chlamydospores and not oidia, as was stated in our first publication. In later cultures, however, I have found oval double-contoured spores in rows at the end of the aërial hyphae. They varied somewhat in size and the largest ones were usually found at the end, giving the whole a somewhat club-shaped appearance. These oval spores were directly attached to one another without any intermediate empty pieces of mycelium. I have never seen any lateral conidia.

In very old pure cultures the spores lose their characteristic shapes and become round or sausage-shaped. They also lose part of their resplendence. When these chlamydospores are brought into fresh media they bud out into new hyphae (see Fig. 31). The spore membrane always bursts at the side.

Gelatine.—Formation of a similar growth occurs on the surface. The aërial hyphae, however, appear late and are not so plentiful. The gelatine beginning with about the tenth day is slowly liquified. Microscopically the growth resembles that on agar-agar. The formation of spores occurs late, however, and is not plentiful.

Raisin-gelatine gives a similar development.

Beef-tea.—Growth at the bottom of the tube in form of irregular flakes that look like pieces of coarse cotton. The growth is first white, later brownish. Microscopically, it consists of a dense felt of septate hyphae of the same appearance as those in other cultures. Formation of air-hyphae and spores was not observed.

Milk is slowly peptonized (in the course of a month or two) without change in reaction. At last one obtains a clear liquid with a thick white scum on the surface, which may show greenish and lilac spots.

Potato.—Abundant growth, very much like that on agar-agar. Early and profuse production of spores.

Carrot and turnip.—Less abundant development and later and less abundant spore formation.

Cultures develop on all media slowly but very well at room temperature (between 15° and 20° C.).

The mould develops very well on all albuminous media (Loeffler's serum, glycerine (6%) serum, ascitic fluid). Protozoon-like bodies were

not observed in any of the cultures even in albuminous media under aërobic and anaërobic conditions.

It was also tried whether a development of the mould into the protozoon-like bodies would take place in a collodion sac that was allowed to remain in the peritoneal cavity of an animal for some time. When the sac was examined five days after its introduction into the peritoneal cavity it contained mould spores only, the mycelium having disappeared completely, but protozoon-like bodies were not found.

I have been able, however, to trace the development of the protozoon-like bodies from the mould after its introduction into the animal body, in Rabbit No. 4. The animal was inoculated into the ear-vein with a culture that contained many spores, but part of the infectious material entered the subcutaneous tissue near the vein. Two days afterwards already a small abscess had developed at the point of inoculation. A microscopic examination of the pus showed that the inoculated mycelial threads had disappeared, but that as yet it contained the injected mould-spores in large numbers and also that a few of them had developed into short hyphae such as are represented in Fig. 28.

On the following (third) day these hyphae began to break up and disappear and had vanished completely on the following day or the day after. With the third day a development of the mould-spores into the protozoon-like bodies began. This development is shown in Fig. 29. As is represented in the figures, the spores either enlarge as a whole (Fig. 29b) and gradually assume the forms of the protozoon-like bodies, or the development is at first uneven. One side of the spore shows a marked bulging (Fig. 29a), but then also in course of time the bodies assume a spherical form and the remnants of the intermediate link disappear. On the fourth day already some of these newly formed protozoon-like bodies had developed into sporulating forms and on the fifth day the sporulating forms were very numerous. On the seventh day the originally injected mould-spores were very few in number and on the tenth day they had disappeared altogether.

In my specimens I have never been able to see any indication of a direct development of mycelium into protozoon-like body, and I do not believe that it ever occurs. In cases in which animals are apparently inoculated with mycelium only, as in the case of Rabbit 2, and in which nevertheless the protozoon-like bodies develop in the tissues, I believe that there must be first a formation of spores and secondarily a development of protozoon-like bodies from them. It may, however, also be that in this case, in the preliminary examination of the pure culture, a few spores were overlooked, which is really more probable.

Inoculation experiments with the pure culture of the mould on guinea-pigs and rabbits were uniformly successful, with the exception of my first experiment on Guinea-pig 4. The culture which was employed in that instance had been developing only a comparatively short time, and the unsuccessful outcome may be explained by a complete absence of spore-formation in the young culture. Otherwise it is difficult to find any reason for it, since in all other cases the infection took very readily.

Two frogs were inoculated, one into the dorsal lymph-space, the other into the peritoneal cavity; they remained healthy and at autopsy did not show any lesions.

In several cases new pure cultures of the mould were obtained by inoculating suitable media with pus from inoculated animals. In all these cases the *pus,* when examined with the microscope, contained protozoon-like bodies in pure culture, and no mycelia, and the *cultures,* which were obtained, mycelia and chlamydospores and no protozoon-like bodies.

I believe that my experiments prove conclusively that the protozoon-like bodies belong to the life cycle of the pathogenic mould, which was cultivated on the artificial media. When the parasite develops in the *animal body* it appears in the form of protoplasmic spheres surrounded by a thick double-contoured membrane and propagates itself by endogenous sporulation; when it develops on the *artificial culture-media* it develops a mycelium, and at the end of the aërial hyphae of this mycelium chlamydospores are formed, which when brought into new artificial media again reproduce the mycelium, and so on. In case, however, these chlamydospores enter the body of susceptible animals, they develop into protozoon-like bodies with endogenous sporulation. These propositions have been proved in two ways. First: Animals have been inoculated with pure cultures of the mould, which did not contain any protozoon-like bodies, and have developed a disease, in the lesions of which the protozoon-like bodies were found in pure culture and no mycelium. From these diseased spots the mould has then again been obtained by cultivation. Secondly: Development of the different stages into one another has been directly traced under the microscope: formation of a mycelium from the protozoon-like bodies and their spores in the hanging drop; development of the chlamydospores into protozoon-like bodies in the subcutaneous abscess in Rabbit No. 4.

CLASSIFICATION.

In regard to the classification of the new pathogenic mould we encounter great difficulties which it has been impossible for me to overcome in a satisfactory manner. I believe that this part of the inquiry belongs rather to the field of botany. I have therefore consulted with several well-known botanists, but they also were at a loss how to place the new fungus, and all seem to agree that our present system of classification of the lower fungi is very imperfect and far from satisfactory in many ways.

The only organism which has at least some points in common with the one described in this paper, is the one that was found by Gilchrist and Stokes [7] in a case of pseudo-lupus. Gilchrist and Stokes observed that blastomycetes in the tissues also form spherical protoplasmic bodies with a thick capsule which, however, multiply by budding and not by endogenous sporulation. When cultivated on artificial media they first show very peculiar budding forms, that are represented on Plates VI and VII, which accompany the article referred to; later one finds a very intricate meshwork of very thin hyphae in the cultures.

I am much indebted to Dr. Gilchrist for giving me the opportunity of studying this parasitic organism. I can only fully confirm Gilchrist and Stokes' observation as far as the growth on artificial media is concerned. In old pure cultures I have regularly found peculiar bodies that seem to develop at the end of the hyphae. They are represented in Figs. 26 and 27. They are about 10 μ in diameter. They consist in a rather thick sometimes double-contoured membranous sac that is filled with small, round, highly refractile granules. These granules do not give the staining reactions of fat. It is possible that they represent a form of endogenous sporulation, but I have not been able to prove this by demonstrating a development of either a new mycelium or the budding forms, that occur in the tissues.

[7] Gilchrist and Stokes. A case of pseudo-lupus vulgaris caused by a blastomyces. *Journal of Experimental Medicine*, 1898, III, 53.

At the meeting of the Chicago Pathological Society on Monday, April 8, 1901, Dr. H. T. Ricketts read a paper on "Blastomycetic (Oidiomycetic) dermatitis and its organisms." As far as I can gather from a short review of his paper, which appeared in the *Philadelphia Medical Journal* of April 27, 1901, he believes that the organisms found in "blastomycetic dermatitis" and also the one isolated from our Case I, although differing in certain respects, all belong to one group of fungi, the oidia. As far as the fungus that is described in this paper is concerned, he has perhaps been slightly misled by our preliminary report, in which it was stated, that oidia had been found in the cultures, yet as far as his contention goes that all these fungi belong together, I agree with him perfectly. A closer investigation, however, has shown that our first statement of the occurrence of oidia was erroneous. The majority of the spore-like bodies are certainly not oidia, but a modification of them—chlamydospores—the characteristic being that in the hyphae the protoplasm is concentrated at certain points in the form of spores, leaving between the spores segments of the original hyphae, which are empty, as is shown in Fig. 25. Following the general laws of oidia and chlamydospores, the chlamydospores formed in our fungus can develop either into a new vegetative form (development of hyphae from them when brought upon new media, see Fig. 31) or under favorable conditions (in our case in the animal body) into "Fruchtkörper" with endogenous sporulation. Following Brefeld's classification of fungi, if I understand correctly, our fungus on account of this constant and typical endogenous sporulation would belong to the ascomycetes.[8]

On account of the difficulties in the way of an intelligent classifi-

[8]Since the above was written, I have had the opportunity not only to study Dr. Rickett's article in the original, but also to see a number of his cultures, which he was kind enough to show me. I am now more than ever convinced that the organisms which he describes and also the one studied in this paper are all very closely related to one another, and belong to a rather badly defined genus interposed between hyphomycetes and blastomycetes. All oidia would belong to the same group and it would perhaps be well to enlarge the meaning of oidium sufficiently to make it cover the entire group, as Rickett in his paper proposes to do.

cation of the parasite, I believe it to be best to reserve the adoption or coining of a generic name for the future, and use the name which has been proposed by Rixford and Gilchrist as a generic name for what they believed to be a protozoon, as a name for the species. Its name then would be *Oidium coccidioides,* or, if there should be objections to the adoption of coccidioides, *Oidium protozoides.*

I have also had an opportunity of studying the blastomycetes that were isolated by Dyer [9] from a case of blastomycetic dermatitis. This organism is certainly entirely different from either Gilchrist and Stokes' or the one described in this paper, growing in all respects like an ordinary blastomyces and causing alcoholic fermentation in sugar-solutions, whereas in the case of the other two parasites I have never seen any evidence of such fermentative action.

LESIONS PRODUCED IN LOWER ANIMALS AND MAN.

A short synopsis of the cases which up to date have been observed in the human being, will serve best to show their similarity in many respects and also to bring out their individual peculiarities.

I. Cases with Primary Cutaneous Infection.

No. 1.—Case of Posadas and Wernicke.—Middle-aged man. First symptom: nodular growths in skin of face and of left thigh resembling mycosis fungoides. Secondary involvement of regionary lymph-glands. Disease lasted seven years. At postmortem miliary tubercles in internal organs.

No. 2. First case of Dr. Rixford and Dr. Gilchrist. Man 40 years old, from the Azores. First symptom: sores on forehead and back of neck. Character of skin lesions: irregular ulcers with fungating masses of granulation tissue containing tubercles. Slowly spreading over face, and infection of other parts of skin. Nine years after first changes in skin involvement of regionary lymph-glands Remittent fever, which lessened after antiseptic treatment of ulcers (fever due to secondary infection with pyogenic cocci?) destruction of both eyes. Death about ten years after first symptoms.

[9] Dyer. Blastomycetic Dermatitis, etc. *American Journal of Dermatology and Genito-Urin. Dis.*, 1900, *No.* 3.

Lesions found at postmortem: Extensive chronic tuberculosis and ulceration of skin. A few small cavities, chronic miliary tuberculosis, old scars and diffuse consolidation of lungs. Chronic tubercular pleurisy and peritonitis. Tubercles in spleen. Multiple abscesses in mesenteric and peribronchial lymph-glands. Chronic tuberculosis of both adrenals. Chronic tuberculosis and suppuration of both testes and prostate. Suppurative necrotizing osteomyelitis of middle of left tibia and head of metacarpal bone of left index finger.

No. 3. Second case of Rixford and Gilchrist. Man 33 years old, from the Azores. First symptoms: pimples on left side of forehead which developed into chronic ulcers. Character of cutaneous lesion: first infiltration of skin, then flat, rapidly growing irregular ulcers. Soon afterwards infection of other parts of the skin. Early involvement of regionary lymph-glands. Metastatic infection of inguinal lymph-gland by way of the blood current. Night-sweats, remittent fever. Duration of disease, three months. No necropsy.

II. Cases with Primary Pulmonary Infection without Cutaneous Infection.

No. 4. *Case* I. Boy 19 years of age, born in the Azores. First symptom: chill followed by pleurisy; later multiple acute arthritis, osteomyelitis of frontal bone. Cough and expectoration. Slight chills and profuse sweating. Duration of disease, three months.

Lesions found at postmortem: Abscesses and diffuse consolidations of both lungs, empyema on left side, purulent infiltration of diaphragm, multiple abscesses and necrosis in retroperitoneal lymph-glands. Miliary abscesses in liver and kidney. Miliary tubercles in kidneys. Multiple foci of purulent osteomyelitis and arthritis.

No. 5. *Case* II. No clinical history; old man, German. Lesions found at postmortem: Old, partly healed lesions in lungs. Chronic tuberculous pericarditis, miliary tuberculosis of spleen and both kidneys, basilar tuberculous meningitis.

III. Case of Apparent Primary Infection of Lungs with Cutaneous Lesions.

No. 6. Dr. Montgomery's case. Male, 21 years of age. German. First symptom: cough ten months before death. Multiple cutaneous lesions appeared two months before death. Character of cutaneous lesions: Large tubers like those of mykosis fungoides, later suppuration.

High remittent fever with night-sweats. Secondary involvement of regionary glands. Marked rapidly progressing pulmonary lesions.

Findings at postmortem: Cutaneous lesions. Large abscess of the size of an infant's head in upper part of right lung communicating with abscess of the neck and the chest underneath the clavicle on the right side. Several smaller abscesses and diffuse consolidation of right lung. Immense abscess of liver. Kidneys and spleen apparently normal.

A comparison of these cases shows in the first place that the duration of the disease varies within wide limits (from three months to nine years) and also that the duration in no way depends on the primary seat of infection.

The clinical symptoms vary very much and of course depend entirely on the organs affected, and on the extent of the lesions in them. Remittent fever and night-sweats seem to be a constant feature of the disease, at least in its later stages. They were present in all four cases of which I found clinical records.

In all cases the disease shows a marked tendency to spread as well by way of the lymph-channels as by way of the blood-current. Case 2, however, proves that this tendency to generalization may manifest itself very late (nine years after the beginning of the disease).

The cutaneous lesions, when they are present, may apparently be quite different in different cases, showing either more the appearance of mykosis fungoides with large tubers and secondary slow ulceration (Cases 1 and 6) or that of a chronic tuberculosis verrucosa cutis (Case 2); or again one observes rapid infiltration of the skin with extensive flat ulceration (Case 3). The differences are due to the more or less rapid progress of the destructive process.

In all organs the disease appears in the form of miliary nodules or miliary, rarely larger, abscesses, which exceptionally, however, may attain a very considerable size (abscess of size of child's head in lung and immense abscess in liver in Case 6).

The disease, therefore, belongs to the infectious granulomata and shows a great deal of similarity to glanders. In fact, when I first saw the specimens of Case 5, I at once thought of glanders. As a name for the disease I should propose *coccidioidal granuloma.*

What is true in these respects of the human cases, also holds good for the disease in guinea-pigs and rabbits, as a study of the detailed accounts of the animal experiments, which are given at the end of the paper, will readily show.

I should like to call special attention to the frequent multiple infections of the bone (Cases 2 and 4, Rabbit 3 [osteomyelitis of some lumbar vertebrae with compression of the cord, osteomyelitis of the parietal bones with compression of the cerebrum]).

The infection of the testicles and prostate in human Case 2 acquires interest when we observe the constant occurrence of suppurative periorchitis in guinea-pigs after intraperitoneal infection. *En passant* I should like to point out that here again we have a new proof that suppurative periorchitis in guinea-pigs is not pathognomonic for glanders, as is maintained by some authors.

Although frequently the lesions have a marked tendency to progressive development, they are chronic in so far as even the abscesses are always surrounded by a considerable amount of cicatricial tissue. Individual lesions may heal spontaneously, as is well shown in Case 5 and in Rabbit 3 (see also Fig. 21). Sometimes the healed, or nearly healed, nodules show calcification. But while this healing tendency may be well marked in certain parts of the body, the disease itself always seems to progress to a fatal termination. At least such has been the case in all human cases, and in all animals that were not killed prematurely or were not cured by surgical interference (Dr. Rixford's cases).

The *histology* of the lesions is of the greatest interest from a general pathological standpoint. In the first place they furnish proof that the typical tubercle (consisting of epithelioid cells and a peripheral zone containing lymphocytes and plasma cells) with giant cells of the Langhans type and central caseation, is not absolutely characteristic of an infection with tubercle bacilli. Our parasite in the human being as well as in animals can produce lesions which histologically cannot be in any way differentiated from the nodules produced by tubercle bacilli (see especially Fig. 7).

In the second place we observe in this disease remarkable transitional stages between nodular lesions on one hand, and chronic miliary abscesses on the other. There are nodules with central abscess formation (see Fig. 18) and chronic abscesses with central caseation of their contents.

Furthermore, sometimes we find large masses of fibrous tissue with numerous very large fibroblasts, few leucocytes and occasional giant cells usually including one or more of the parasites. These masses in their structure bear a certain resemblance to some tumors of the class of fibrosarcomata or fibromyxosarcomata.

It may appear extraordinary that one parasite should be able to produce lesions of such entirely different aspect. A careful study of the tissues, however, has convinced me that these differences are largely dependent on the stage of development in which the parasite is present. The adult forms are found in the less acute lesions, whereas the abscesses always contain sporulating forms, often in very large numbers. A study of the illustrations given by Rixford and Gilchrist seems to prove that this holds good for their cases also. It is true that occasionally one finds a sporulating form in a nodule, as is represented in Fig. 23, but in all these instances the capsule which surrounds the spores is intact, or seems to have burst only recently. It seems then that only after the bursting of the capsule the substances which produce the suppurative process gain access to the tissues.

The fact that the sporulating forms contain a substance which has a strong positive chemotactic influence upon the polymorphonuclear leucocytes is also evidenced by their very frequently entering into them soon after the capsule has burst, and often collecting in them in large numbers. In our Fig. 16, a polymorphonuclear leucocyte is just entering, as it were, the capsule through a small opening, whereas our Fig. 13 and Fig. 8 in Rixford and Gilchrist's Plate XLI show empty cells full of polymorphonuclear leucocytes. This allows us to understand also why in Case 3 with very rapid course and much suppuration sporulating forms were so plentiful.

That part of the poisonous substances which are elaborated by the parasite are absorbed and cause severe general disturbances in demonstrated by the fever and the night-sweats in the human cases. It even seems that sometimes the absorbed toxins are powerful enough to produce focal necroses in the liver, which are so common in other infectious diseases. At least this is the only interpretation that I can give to the peculiar lesions which were found in the liver of Rabbit 2, and are represented in Fig. 15.

I am doubtful whether the slight diffuse cirrhotic changes in the liver of Rabbit 3 are also due to this general intoxication, or whether they constitute an accidental complication.

CONCLUSIONS.

From the preceding I derive the following conclusions:

1. The disease which formerly has been described as a form of protozoon-infection is due to an infection with a pathogenic fungus.

2. The infection may primarily be either a cutaneous or a pulmonary one.

3. The lesions produced by this fungus fall under the general head of infectious granulomata and consist partly in nodules resembling altogether those produced by the tubercle bacilli and partly in chronic abscesses.

4. The adult forms of the parasite are more apt to produce nodules, the sporulating forms abscesses.

5. The fungus is pathogenic for dogs, rabbits and guinea-pigs probably other animals also, and in them produces lesions very similar to those which we encounter in the human being in this disease.

6. Supurative periorchitis in guinea-pigs is not pathognomonic for glanders.

In concluding I should like to express my obligations to Dr. H. C. Moffitt, who gave me the opportunity to examine Case I, and who allowed me to make use in this paper of the first animal and culture experiments which he performed together with Dr. May Ash.

Since finishing this paper I have seen two more cases of this peculiar affection. One was the unpublished case of Drs. Montgomery and Taylor, mentioned at the beginning of the paper.[10] It was a very typical one with primary cutaneous lesions resembling chronic hypertrophic lupus. With these lesions the patient suffered for a considerable time before he died of an acute generalized miliary (pseudo-) tuberculosis produced by the dissemination of the fungi by way of the blood-current. This case closely resembled Case 2 of the former series. Through the courtesy of Drs. Taylor and Ryffkogel I was able to investigate the case thoroughly before and after death and confirm my former findings in regard to the nature of the parasite and the character of the lesions. The second case was more unusual. The patient, who was under Dr. Morton's care at the German Hospital in San Francisco, was a Japanese section hand employed by the Santa Fe Railroad. He had a chronic ulcer of the right foot just below the ankle on the inside. It was fairly regular, from 3 cm. to 4 cm. in diameter, and covered with a thick brown scab. The edges were very slightly elevated and reddened, the surrounding skin being perfectly smooth and very nearly normal. There was hardly any discharge. The femoral and inguinal lymph-glands became involved, were removed and sent to me for examination. They looked exactly like chronic tubercular lymph-glands with very extensive dry, diffuse caseation. In sections from these lymph-glands, which otherwise presented all the microscopical changes produced ordinarily by chronic tuberculosis, the parasites were discovered; later they were also demonstrated in the primary lesion. Cultures in hanging drops were made with the usual result. After removal of the original ulcer the patient was discharged in an improved condition but not completely cured. There was one fairly large lymph-gland on the right side of the neck, the removal of which was deemed inadvisable. No history, unfortunately, could be obtained from the patient, who spoke Japanese only.

The last case is particularly interesting on account of the unusual

[10] Now published l. c.

appearance of the primary cutaneous lesion and also on account of the extensive dry diffuse caseation in the extirpated lymph-glands.

DETAILED DESCRIPTION OF HUMAN CASES AND ANIMAL EXPERIMENTS.

CASE I.—A. L., aged 19 years; farm laborer; born in the Azores. Entered Ward C, City and County Hospital, January 26, 1900. Died Feb. 6, 1900.

Past History.—Unknown.

Present Illness.—Began eleven weeks ago with a chill. Had been riding in the rain after cattle. Went to bed, and after a few days left pleura was tapped, and one gallon (?) clear fluid obtained. Had pain in left side and back. Three weeks after beginning of illness went to St. Luke's Hospital. There had irregular fever, at times reaching 104.4°; nosebleed. Abdominal tenderness; diazo reaction present; no Widal reaction (two examinations). Leucocytes 7000 in cmm. Movements at first pea-soup-like in character, later solid. Dullness over left apex, later at left base. Tapped January 23, three days before admission to City and County Hospital, with negative result.

Four weeks before entrance (about January 1) joint trouble began. At first there was pain below the left patella along the tendon. One week later left knee-joint swelled. Then right knee, right shoulder, both elbows and wrists swelled, and grew red and tender. There were no remissions of pain or swelling. For three weeks before entrance great pain in frontal region, which it was impossible to relieve.

Profuse sweating. A large gland developed in left supraclavicular fossa, a week before entering City and County Hospital, also a fluctuant swelling over left eye. Cough with muco-purulent and occasionally blood-streaked expectoration. No tubercle bacilli in sputum.

Examination (January 27).—Emaciated, yellowish pallor, flushed cheeks. Limbs helpless, due to swelling of joints and pain. Right wrist swollen, red and tender. Right elbow and shoulder red and painful. Both ankles and feet swollen, tenderness over small bones of foot, marked on the left. Both knees swollen and tender. Pain marked along patellar tendons and over crests of tibiæ; evident periostitis of left tibia, less distinct on right. Over left eye a fluctuating swelling, the size of a walnut, starts from frontal bone and is very tender but not reddened.

Long eyelashes, pupils normal. Tongue tremulous, not much coated. Rales heard in mouth. Small glands in submaxillary fossæ and above right clavicle. Large gland the size of walnut over left clavicle. Below

this, reaching downward to the pleural space, is an abscess the size of a lemon. General icterus.

The lower intercostal spaces on both sides bulge. Dullness above both clavicles, more marked on left side. Dullness on right side below clavicle, reaching outward to axilla. Dullness in right axilla. Lung almost tympanitic anteriorly from 1st to 4th rib. Dullness along the 4th rib; deep liver dullness at 5th interspace. Left lung dull over its entire anterior surface; absolutely flat in axilla. On auscultation loud bronchial breathing at right apex; a few rales. A few mucous rales at base. On left side bronchial breathing at apex. Anteriorly coarse and fine, dry and moist rales. Heart large, no alterations in sounds.

Specific gravity of urine is 1020; no albumin; no sugar; indican present; diazo reaction present.

Plantar reflexes normal.

Jan. 31. Leucocytes 17,000 in cmm.

Feb. 4. Slight chills. Profuse sweating.

Feb. 6. Large mucous rales at the base of the left lung.

Tubercle bacilli were not found in spite of repeated examinations of the sputum. The patient showed progressive wasting and died Feb. 6, 1900.

The *autopsy,* by Dr. Moffitt, showed:

Right lung: Irregular, nodular consolidations with necrotic centers from the size of a shot to 1 cm. in diameter. The tissue between them was in great part atelectatic; in the middle lobe were several abscesses with thick abscess-membrane. Left lung: Acute broncho-pneumonia and chronic interstitial pneumonia with abscess-formation; also nodules like tubercles as on right side. There was a large abscess partly in the mediastinum, partly in the left lung.

The pillars of the diaphragm were infiltrated with pus, and there were necroses and abscesses in the retroperitoneal lymph-glands. The right pleura was free; in the upper part of the left pleura an encapsulated empyema was found; in the lower part adhesions.

The spleen was enlarged and softened; in the pulp were some ill-defined yellow patches.

The kidneys showed cloudy swelling; in both of them were miliary nodules with central caseation or miliary abscesses with thick abscess-membrane. The nodules and abscesses were mostly in pyramids.

In the liver were cloudy swelling and fatty infiltration, but no visible nodules.

There were purulent ostitis and periostitis in the frontal bone over the

left eye and in the upper part of the left tibia, and suppuration in the affected joints. Necroses and abscesses in regionary lymph-glands.

Microscopic Findings.—Lungs (see Figs. 2, 3, 4).—Sections of the lungs are full of irregular, miliary and larger, often conglomerate nodules. Many of them seem to have originated in the perivascular and peribronchial connective tissue, whereas some seem to have formed in the pulmonary tissue proper, but even those which arise in the peribronchial or perivascular connective tissue, have pushed their way into the adjoining pulmonary tissue. The structure of the majority of the nodules is the following (Fig. 4): The air cells in the affected area are filled with a cellular exudate which consists mostly of epithelioid cells and lymphocytes imbedded in a meshwork of fibrin. Many of the lymphocytes show the characteristic appearance of plasma cells. Among the cells there are also a few polymorphonuclear leucocytes. One also finds large giant cells, many of them with a peripheral arrangement of their nuclei. The centers of the nodules often show extensive caseation (not present in figure) with much nuclear debris in a necrotic, finely granular material. There is a little, but not much, fibrin between the cells and in the caseated matter. In a few places a few connective-tissue fibrils can be seen between the epithelioid cells. In other places (Fig. 5) abscess-like collections of polymorphonuclear leucocytes may be seen in the middle of the caseous areas. Many of the polymorphonuclear leucocytes, especially those in the middle of the abscesses, show signs of beginning disintegration (pyknosis, karyorrhexis). In the pulmonary tissue between the nodules the air-cells are partly collapsed, filled with coagulated oedema and large desquamated epithelial cells with some lymphocytes and a very few polymorphonuclear leucocytes. In no place, even in the nodules with more chronic appearances, do the septa participate much in the diseased process. They usually show only a moderate infiltration with lymphocytes and plasma cells. In a few spots the lesions show a more acute character throughout. In these places all the air-cells are filled with polymorphonuclear leucocytes, desquamated epithelium, oedematous exudate or a little fibrin. In the middle of such areas there is usually evidence of beginning degeneration and nuclear fragmentation of the leucocytes.

Parasites are present in large numbers in all stages of development. In the tubercle-like nodules one finds mostly young and adult forms (see Fig. 4), almost invariably enclosed in giant cells. In the abscesses there are a very large number of sporulating forms (see Fig. 2), with very thick membranes, which are often prickly on their outer surface. In most of them the spores have already developed into young forms. In

many places one can see that the polymorphonuclear leucocytes have entered the interior of the capsule in which the spores lie through the opening formed by its bursting.

The tissue is in a bad state of preservation. Everywhere one finds very many large bacilli in chains, resembling morphologically anthrax bacilli. They do not show any constant relation to the lesions in their position, and are most abundant in the blood-vessels so that one seems justified in assuming that they are organisms of putrefaction (they did not develop in any of the cultures). Mycelia were not found in any place.

Kidneys (Figs. 5 and 6).—Very many miliary and submiliary chronic abscesses are present. The contents of the abscess-cavities consist of polymorphonuclear leucocytes, some lymphocytes, and a considerable number of epithelioid cells. Around the abscess cavities there are layers of granulation tissue with many epithelioid cells, more peripherally lymphocytes (mostly plasma cells). Giant cells are present, but are not very numerous. The largest abscesses show central necroses. In these necrotic areas dead polymorphonuclear leucocytes, etc., are fused together into a dense granular substance which resembles very much caseous material, and is full of nuclear debris. In other places (Fig. 6) there are more tubercle-like nodules, which consist largely of epithelioid cells. In the periphery of these nodules one finds many lymphocytes and plasma cells. There may be some giant cells and in spots beginning caseation. Extensive necroses are seen in the epithelial cells of the convoluted tubes and ascending loops of Henle.

Parasites are numerous, and are found in all stages of development. In the middle of the abscesses there are many sporulating forms (Fig. 5) or spore membranes enclosing many young parasites. No other bacteria, no mycelium.

Liver (Fig. 7).—Very small submiliary nodules, which consist largely of epithelioid cells and lymphocytes (many plasma cells) occur. The lymphocytes and plasma cells are mostly in the periphery of the nodules. Among the epithelioid cells one may find a few large giant cells of Langhans' type. The centers of the nodules are often caseated. Moderate numbers of young and adult *parasites* appear, almost invariably in giant cells. In a few of the nodules sporulating forms, surrounded by a small gathering of polymorphonuclear leucocytes, are encountered. No other bacteria, no mycelium.

Spleen (Fig. 8).—Many submiliary abscesses with some epithelioid cells in the periphery are present. In some of them are apparent central

necrosis and formation of granular or fibrinous deposits with much nuclear debris. Many *sporulating forms* are seen in abscesses. In a few spots are tubercle-like nodules consisting of epithelioid cells, lymphocytes, and giant cells, and containing a few young or adult forms. No other bacteria, no mycelium.

Stomach.—Some atrophy of mucous membrane, superficial (post-mortem) necrosis, otherwise normal.

Sections from all organs were stained for tubercle bacilli and carefully searched for them, but with negative result.

Parasites were also found in abundance in the pus from the abscess over the left eye. Here they were associated with staphylococci and streptococci. In the left pleura (empyema) they were also present in large numbers together with pseudo-diphtheria bacilli.

Case II (Service of Dr. Hirschfelder).—G. B., German, admitted May 8, 1900, from County Jail No. 2.

In spite of all endeavors it has not been possible to obtain any information in regard to his former history.

Patient entered hospital in an unconscious condition, breathing stertorously. Pupils small, reacting equally to light. Head turned to right. Slight paresis of muscles of right side of face. Muscles of extremities rigid. No pulmonary dullness. Outline of cardiac dullness normal; tones clear; pulse full, regular, 96. Respirations 40. Sensation evidently not impaired. When forehead is pricked with pin patient slowly raises hand to head. With exception of paresis of right side of face, no paralysis or paresis can be made out. Voids urine and feces involuntarily. Urine removed by catheterization. Acid, no albumin, no sugar, no diazo-reaction, no indican.

May 9. Patient somewhat brighter. Evidently understands what is said. Puts out his tongue slowly when told; closes and opens his eyes when told. Drinks when cup is held to his lips.

May 10. Condition unchanged. No dullness over lungs.

May 11. Condition about the same. Patient still unable to speak.

May 12. Same.

May 13. Same.

May 14. Patient died at 2.40 P. M.

Autopsy Record.—96. G. B. Aged 50 years. Laborer. German. Died May 14, 1900, 2.40 P. M. Autopsy May 16, 1900, 3 P. M.

Moderately strongly built, fairly well nourished man. No enlargement of superficial lymph-glands. Abdomen moderately distended. No

lesion on external genitalia. Diffuse brown discoloration of the skin. Slight cyanosis of both ears. Beginning green discoloration of abdominal wall. A few drops of slightly bloody fluid in recto-vesical pouch. Diaphragm, 5th rib on both sides. Thorax well arched, symmetric. A few drops of slightly bloody fluid in right pleural cavity. Both layers of the pericardium attached to one another by moderately firm adhesions; pericardial space entirely obliterated.

Heart.—Post-mortem clot in pulmonary artery. Valves normal on both sides; slight atheroma of aorta. Cavities of heart normal size. Muscle soft and flabby. At the base of the heart the pericardium is much thickened. In spots the newly formed cicatricial tissue is infiltrated with a little purulent fluid. In other places there are a few, just visible, greyish-white or yellow nodules.

Lungs.—A few stringy adhesions at the apex of the left lung. Pleura over left lung otherwise normal. The lung itself is a little cyanotic. There is a little emphysema along the free margin. A little muco-pus in bronchial tubes. The mucous membrane of the bronchi is slightly reddened. At the apex near the adhesions there is a small black scar. Slight thickening of pleura at right apex. Otherwise right lung same as left.

Spleen.—A little large, 13 x 11 x 4.5 cm. It is soft. The cut surface has a brick-red color and the markings are indefinite.

Both *adrenals* normal.

Left kidney a little large. Surface studded with greyish-white submiliary nodules. The nodules are solid and some of them are surrounded by a hyperæmic zone. A few similar nodules in cortex on cut surface. Pelvis of kidney normal. Right kidney is in the same condition as the left one.

Appendix is of normal caliber and length. It is found curled up in a little peritoneal pouch, which is situated on the median side of coecum, directly under the lower end of the ileum and within the mesentery. This pouch is completely shut off from the rest of the peritoneal cavity by a thin transparent membrane. The *sigmoid flexure* is fastened to the brim of the pelvis by some white stringy adhesions.

A little turbid urine was found in the bladder.

Bladder, prostate, seminal vesicles, rectum, right *testis* normal.

No enlargement of *mesenteric glands.*

Bile-duct is open.

A little dark brown mucus in *stomach.* Extensive post-mortem digestion in fundus. Atrophy of mucous membrane in pyloric region.

Pancreas normal.

Liver very much decomposed and full of gas bubbles. Gall-bladder and contents normal.

Thick white coating on upper surface of *tongue.*

Pharynx filled with muco-pus. *Tonsils* slightly enlarged.

Oesophagus, larynx, trachea, thyroid, thoracic aorta normal.

Some of *peribronchial lymph-glands* are enlarged and show numerous black spots on cut surface.

Longitudinal sinus contains a few post-mortem clots.

Much oedematous fluid and gas in *pia mater.*

Venous sinus at basis of skull normal.

The pia mater at the *basis of the brain* and more especially in the Sylvian fissures is diffusely infiltrated with pus and full of submiliary yellow nodules. Large numbers of similar nodules are found at the bottom of the longitudinal fissure of the brain. A little turbid fluid in both lateral ventricles. On both sides of the septum the ependyma is studded with just visible, translucent, greyish-white nodules. The substance of brain is a little oedematous and anaemic, otherwise apparently normal.

Anatomical Diagnosis.—Small scars in both apices of lungs. Pleuritic adhesions. Purulent infiltration and (pseudo) tuberculosis of pericardium at basis of heart. Chronic pericarditis with complete obliteration of pericardium. (Pseudo) tuberculosis of meninges, spleen and both kidneys. Ependymitis chronica nodosa.

Microscopic Findings.—Brain (basis, Figs. 9 and 10).—Pia mater very much thickened, hyperæmic; it contains many small abscesses. Most of them are more or less spherical, others more irregular in form. At their central portions one finds irregular masses consisting of fibrin, fragments of nuclei, bodies and fragments of small round cells, some intact and some broken-down blood-corpuscles, a few intact cells, most of which are polymorphonuclear leucocytes. Further outwards follows a zone in which there is less fibrin, forming long slender fibers woven into a delicate network. In the meshes are many polymorphonuclear leucocytes and a few lymphocytes. In the periphery of the abscesses one encounters mostly lymphocytes, many plasma cells and large mononuclear cells, which often enclose in their bodies lymphocytes, or one or more of the few polymorphonuclear leucocytes which are also present. Other cells of this kind are very large and have a vacuolated appearance, very much as if they had been loaded with fat. There is no evidence of a formation of connective-tissue fibers between the large mononuclear cells. In some abscesses one finds large giant cells, some of which are of the Langhans type, whereas in other

cells the nuclei are situated in the center. In a few spots the character of the lesion differs from what has just been described. In these places one finds nodules consisting almost entirely of large, more or less irregular, mononuclear cells, with lymphocytes and plasma cells. The center of them is frequently necrotic. These nodules, which resemble very much recent tubercles, sometimes contain giant cells. The tissue between nodules and submiliary abscesses contains much fibrin; in many places there are hæmorrhages. It shows a diffuse irregular infiltration with all the different form of cells mentioned, with the exception of giant cells. The blood-vessels are hyperæmic; the smaller ones show marked marginal disposition of leucocytes. A few smaller ones are obliterated by thrombosis. The thrombi contain many leucocytes of different forms. Smaller, not occluding, thrombi of a similar kind are present in some of the larger blood-vessels.

The *parasites* can be found in the abscesses and nodules, but they are present in small numbers only—apparently one or two in each abscess or nodule. In most cases one finds empty shells; in other instances are found sporulating forms with many oval spores in a thick double-contoured membrane. The spores stain hardly at all with any of the dyes that were used (hæmatoxylin, eosin, methylene blue, Van Gieson's stain, carbolfuchsin). Spore-like bodies may also occasionally be found in the surroundings of an empty burst shell. Quite often these empty shells are filled with leucocytes, mostly polymorphonuclear forms. Only rarely one finds young forms. The parasites are often enclosed in giant cells.

No tubercle bacilli nor any other bacteria are found.

Testicle.—No changes.

Spleen.—Slight brown pigmentation. Pulp rich in large mononuclear cells and plasma cells. In one Malpighian body is a submiliary nodule consisting of epithelioid cells.

Kidney.—The nodules consist of a peripheral layer of connective tissue. Inside of this layer of connective tissue there is a layer of tissue consisting mostly of large irregular mononuclear cells, and also some capillaries. The rest of the cells are lymphocytes and plasma cells. In this layer there are several giant cells mostly of the Langhans type. The center of the nodule is formed by a necrotic mass, in the periphery of which one can recognize the former structures in outline. The center is structureless and finely granular. In parts of the necrotic material one finds irregular fragments of nuclei. Almost all epithelial cells in the convoluted tubules and in descending loops of Henle are necrotic.

Stomach.—Marked atrophy of mucous membrane and necrosis of upper layer.

Pericardium at basis of the heart (Fig. 7).—Marked thickening of both layers by development of fibrous tissue. Between the basis of the aorta and the thickened pericardium there are isolated nodules which resemble very much tubercles. They also occur in small groups. They consist of epithelioid cells and lymphocytes. The lymphocytes are more numerous in the periphery and many of them have the appearance of plasma cells. Among the epithelioid cells there are large giant cells of the Langhans type. Some of the nodules show central caseation, in others a fine reticulum of connective-tissue fibers is visible between the epithelioid cells. In one spot the center of a small nodule is completely disintegrated, and the space produced by the destruction of the tissues is filled in with polymorphonuclear leucocytes and some lymphocytes. Between the nodules and outwards from them there is a very thick layer of dense scar tissue, in which there are a considerable number of "Mastzellen." In the neighborhood of the pulmonary artery the vasa vasorum are surrounded by a cellular tissue consisting largely of lymphocytes and fibroblasts. A small vein which runs through the granulation tissue is partly obstructed by a thrombus which contains many polymorphonuclear leucocytes. No parasitic organisms in thrombus.

Parasites are found in considerable number in nodules at the basis of the aorta, mostly young and adult forms; in places where a healing tendency is marked one often finds empty shells. No sporulating forms were seen. No tubercle bacilli and no other bacteria except clumps of short pole-staining bacilli in some of the blood-vessels. No parasites in the granulation tissue in the adventitia of the pulmonary artery.

Lungs.—A section from a small scar near the apex of one lung contains a thick mass of scar tissue with hyalin degeneration and central necrosis; no parasites; clumps of bacilli in some of the blood-vessels. A section from the lower lobe shows hyperæmia and partial collapse. Bacilli in bronchioli and in some of the blood-vessels.

In a section from a large scar the pulmonary tissue is entirely collapsed; septa somewhat thickened by formation of granulation tissue in them; many giant cells in collapsed air spaces. Large areas of granulation and cicatricial tissue. In granulation tissue nodules of epithelioid cells with giant cells; some show central caseation. In one nodule complete disintegration of center with formation of small cavity which is filled with albuminous detritus and nuclear debris.

Parasites (adult forms and empty shells) in nodules; no sporulation forms; no tubercle bacilli or other bacteria.

Appendix.—Atrophy of mucous membrane. Thickening and induration of submucosa.

GUINEA-PIG No. 1 (Drs. Moffitt and Ash). Feb. 7, 1900.

Piece of lung inserted under skin. Killed April 20. At the point of inoculation a thick scar had developed. In the scar there were a few just visible yellow spots, and in them a few adult forms of the protozoon-like bodies were found. No lesions in regionary lymph-glands or internal organs.

GUINEA-PIG No. 2, male (Drs. Moffitt and Ash). February 10, 1900.

Particles of lung of Case I suspended in normal salt-solution injected into peritoneal cavity; about end of second week, the animal shows loss of customary activity. On or about February 26, animal showed a well-marked orchitis and swelling at the site of inoculation, with gradual emaciation. The guinea-pig was killed with chloroform on March 2.

Findings at Autopsy.—Suppuration around both testicles; enlargement and inflammation of inguinal glands; white miliary and submiliary nodules in spleen, diaphragm and lungs. Fresh specimens from the different organs showed the protozoon-like bodies in different stages of development; no mycelia. Cultures from pus near testicle and cultures from the abdominal cavity gave the same mould that had been recovered from pleura and spleen of Case I. A culture from the blood remained sterile.

Result of *microscopic* examination:

Lungs.—Large areas in which the septa are moderately thickened by an infiltration with lymphocytes (mostly plasma cells). The air-spaces are partially collapsed. They contain enlarged and desquamated epithelial cells, some giant cells, in spots a few polymorphonuclear leucocytes and a little fibrin. *Parasites* were present in moderately large number, mostly mature forms. A few sporulating forms and some burst spore shells.

Inguinal Gland.—No changes.

Kidney.—No changes.

Spleen.—In the capsule small nodules consisting of cicatricial tissue with a few plasma cells in the periphery. *Many adult forms* in this tissue.

Heart.—A small thrombus between trabeculae carneae at the bottom of the left ventricle. In spots the thrombus shows irregular groups of polymorphonuclear leucocytes.

Cerebrum and *cerebellum* normal.

Testicle.—On the surface of the testicle has been formed an irregular thick layer of a rather cellular cicatricial tissue, in which there are few capillaries. In it there are small irregular patches of more cellular tissue. In spots these patches consist of epithelioid cells and contain giant cells of the Langhans type; in short, they resemble very much tubercles (Fig. 11). Sometimes giant cells containing parasites are directly imbedded in the cicatricial tissue. In most places, however, there are smaller and larger irregular abscesses which are in great part filled with polymorphonuclear leucocytes, and surrounded by a thin layer of epithelioid cells. Parasites are present in large number. The young and adult forms are mostly enclosed in the giant cells in tubercles and cicatricial tissue. Sporulating forms are found in the abscesses. The old sporulating forms have a rough outer surface. The minute irregular projections which one finds on their outside stain with Gram's method.

Diaphragm.—Large nodules on lower surface. Nodules consist largely of fibroblasts with small strands of connective-tissue fibrils between them. In spots there are groups of plasma cells. In one place there is a small nodule that consists almost entirely of fusiform cells with radial arrangement. The center of the nodule is formed by a *sporulating form* in which the spores are almost ready formed. In the fibrous tissue there are many adult *parasites*, mostly enveloped by a thin protoplasmic layer of large connective-tissue cells which frequently have several nuclei. Large typical giant cells are scarce.

GUINEA-PIG No. 3, male. March 2, 1900.

Inoculated with the pus from one of the testicles of Guinea-pig 2, intraperitoneally. In ten days there developed swelling of the testicles, later the symptoms subsided somewhat. The animal was killed April 7, with chloroform, and presented similar but more extensive lesions than the second guinea-pig. In addition to the nodules in spleen, diaphragm and lungs there were others in the peritoneum near the point of injection. The omentum was rolled up and transformed into a mass of hard white tissue with just visible yellow dots in it. Again the microscopic examination of fresh specimens showed the protozoon-like bodies in pure culture, but no mycelia, and tubes inoculated from testicle and omentum developed pure cultures of the mould.

Microscopic Examination.—Omentum is attached to pancreas by thick layer of dense cicatricial tissue. The omentum itself is transformed into an irregular mass of cicatricial tissue, which contains numerous abscesses. The cicatricial tissue is rich in cells; the majority of them are fusiform or more or less star-shaped connective-tissue cells. Between the cells there are bunches of connective-tissue fibrils. There are also many large polynuclear giant cells present, most of them of the Langhans type. In addition one finds a considerable number of lymphocytes. Apparently no capillaries.

The abscesses are filled almost entirely with polymorphonuclear leucocytes, but there are a few lymphocytes and a few irregular epithelioid cells in them. Of the latter there is a thin layer on the wall of the abscesses. In the centers of some of the abscesses one observes beginning disintegration of the polymorphonuclears, and beginning formation of fibrin.

Parasites.—Many young and adult forms are scattered through the cicatricial tissue, all enclosed in giant cells. Several show evidence of degeneration—a small amount of protoplasm, partial collapse of membrane (Fig. 17) and vacuolization. In the abscesses are only a few young and adult forms, but many burst spore shells. Of five sporulating forms, four were situated in abscesses. In those in which the spore membrane had burst, polymorphonuclear leucocytes have penetrated into the interior. In the sporulating form, which was not in an abscess, the protoplasm had divided, but the spores were not ready formed and the membrane was unopened. The small portions of protoplasm out of which the spores later form, stain very well, the spores themselves very badly, or not at all. No other bacteria, no mycelium.

The lesions in and around the *testicle* (Figs. 12, 13, 14, 16, 17) have very much the same microscopic appearance as those found in the omentum. The amount of suppuration is a little larger, and there is more evidence of destruction of polymorphonuclear leucocytes in the middle of the abscesses.

There is also a considerable amount of fibrin in these necrotic areas. Several *sporulating forms* and many burst shells are found in the abscesses. In the granulation and cicatricial tissue around the abscesses are several young and several adult forms (some of them in a degenerated condition) in giant cells.

Adrenal.—Normal.

Liver.—In one spot is a very small nodule in the periportal connective tissue. The nodule consists of fibroblasts and lymphocytes. There is one giant cell in it. *No parasites.* A complete series of sections from the nodule was, however, not obtained.

Lung.—A few small patches in which the septa are thickened by an infiltration with lymphocytes (mostly plasma cells) and fibroblasts. The air-spaces are collapsed. The epithelium is swollen and partly desquamated. In one of the air-cells several young *parasites* in a giant cell.

Diaphragm.—On lower surface a small nodule consisting of fibroblasts and mostly plasma cells. No *parasites* found (not a complete series).

Kidneys and *pancreas* normal.

Spleen.—One very small nodule consisting largely of epithelioid cells. It contains a few polymorphonuclear leucocytes and giant cells. No *parasite* found (not a complete series). In fresh specimens, however, a few adult forms were demonstrated.

GUINEA-PIG No. 4.

About 1 c.cm. of a suspension of a third generation from a pure culture of the mould on agar-agar was injected into the peritoneal cavity. The culture had been growing a comparatively short time only. The guinea-pig was killed about four weeks later. No lesions were found at the autopsy.

GUINEA-PIG No. 5. October 23, 1900.

An emulsion from a pure culture of the mould, which contained many spores, was injected subcutaneously. On November 2, marked swelling at the point of injection with central ulceration was noted. The ulcer was covered with a thick brown scab, underneath which there was a considerable accumulation of thick creamy pus. Killed December 7, 1900. The condition had not changed materially. There was little loss of weight.

At the postmortem, which was performed immediately, a small ulcer was found at the point of inoculation, which was covered by a thick brown scab. Underneath the scab there was a small accumulation of thick creamy pus. The bottom of the ulcer was formed by a thin layer of red granulation tissue. Underneath the granulation tissue there was some white cicatricial tissue. The muscles of the abdominal wall were not involved in the process. The inguinal glands were enlarged on the left side (side of inoculation). The largest one had the size of a small split-pea. In the center of this lymph-gland there was a comparatively large abscess-cavity which was filled with thick, light-yellow purulent material. In one place the abscess had perforated through the skin.

Spleen.—Slightly enlarged.

A few just visible nodules in the *lungs.*

The other organs did not show any microscopic changes.

In the pus from the ulceration a large number of protozoon-like bodies in different stages of development were found during the lifetime of the animal and also at the postmortem. Mycelia were not present.

GUINEA-PIG No. 6. October 23, 1900.

Inoculated with the same emulsion intraperitoneally.

November 2. Marked swelling of both testicles with reddening of the skin over them. The animal was killed on December 2. At the point of injection a small ulcer had formed, from which there was a scanty purulent discharge.

The tissues around the ulcer were transformed into a hard, partly greyish-white, partly yellowish mass. The change extends down to the peritoneum, which over the diseased area in the abdominal wall is considerably thickened, white in color and retracted in the form of a star-shaped scar. In the *parietal peritoneum* there are several projecting yellowish-white nodules. The largest one has the size of a millet-seed. The peritoneum near these nodules shows a white discoloration. Numerous nodules and extensive diffuse infiltrations are found in the diaphragm. One nodule with a small central defect is situated at the apex of the bladder.

The *omentum* is retracted and forms a thick, irregular, hard, nodular mass along the larger curvature of the stomach. On the left side it adheres to the abdominal wall. On cross section one finds in the partly white, partly greyish tissue many submiliary abscesses.

The *spleen* is adherent to posterior abdominal wall and laterally to the omentum. It is slightly enlarged and contains a few submiliary nodules.

In both *kidneys* there are a few greyish-white nodules.

Both *testicles* are about three times the normal size, and adherent to the skin. In front between testes and skin there are large, irregular abscess-cavities which are filled with thick creamy pus. On the left side the abscess has perforated. The testicular tissue is almost completely destroyed. In its place one finds greyish-white granulation and white cicatricial tissue.

In the *liver* there are a few nodules. The largest ones have the size of a millet-seed and show a central yellow discoloration. Many submiliary grey nodules in the *lungs*.

Heart normal.

A few small nodules in the *pancreas*.

On both sides of the spinal column there is a thick layer of granulation and cicatricial tissue in and underneath the peritoneum.

One *inguinal gland* on the opposite side from the point of injection is slightly enlarged.

Protozoon-like bodies were found in fresh specimens from the nodules and the abscesses. No mycelia.

RABBIT No. 1, large male. March 21, 1900.

Into vein of ear was injected .75 c.cm. of a milky suspension of a pure culture grown on sugar-agar-agar (2% glucose) with contained very many spores.

May 5. Rabbit much emaciated, very weak. Killed.

Very many submiliary white nodules in lungs, a few just visible nodules in spleen and kidneys.

Microscopic Examination.—Liver.—Very few, very small nodules consisting of epithelioid cells and lymphocytes with central giant cells. The nodules are situated in the periportal connective tissue.

Kidney (Fig. 22).—Submiliary nodules in which there is a gathering of epithelioid cells, some polymorphonuclear leucocytes and plasma cells in the spaces between the uriniferous tubules. Some such cells are also found inside of some of the tubules. In the center of the nodules the epithelial cells fill the lumen of the tubules completely and fuse together into large multinucleated protoplasmic masses. In some of these latter some of the nuclei have undergone degenerative changes; they shrink, become irregular in outline and stain very intensely throughout (pyknosis). Nevertheless they show a well-marked, very deeply staining nucleolus. The diseased areas are always situated near a small artery. There is a slight production of connective-tissue fibrils in them; no sign of caseation or suppuration. In some of the tubules one finds casts containing remnants of polymorphonuclear leucocytes and epithelioid cells. The nodules contain many young and adult *parasites*. Sporulating forms were not found in spite of careful examination of many sections and fresh teased specimens. The protoplasm of most of the parasites is finely vacuolated. Their membrane is exceptionally thin. There are few degenerating forms and a few empty shells. In some places the parasites are situated in enlarged epithelial cells.

Spleen.—One small area consisting of fibroblasts with some connective-tissue fibers between them. There are also some lymphocytes (mostly plasma cells) and many large giant cells. Several young and adult *parasites* are found enclosed in the giant cells.

Lungs (Fig. 20).—Large nodular areas in which the septa are thickened on account of marked infiltration with lymphocytes (mostly plasma cells). The air-spaces are partly collapsed. The epithelium is swollen, and there are signs of proliferation. On account of the large size of the epithelial cells some of the air-spaces have a gland-like appearance. In other air-spaces the desquamated epithelial cells have become fused together into large giant cells. It is possible to recognize by the different appearance of their nuclei, that sometimes epithelioid cells also participate in the formation of such giant cells. In some of the giant cells some of the nuclei are pyknotic. In one especially large nodule the center is formed by detritus which is full of nuclear debris and remnants of polymorphonuclear leucocytes.

Parasites are present in moderate numbers; young and adult forms; no sporulating forms. Mycelia were not found in sections or in fresh teased specimens. No tubercle bacilli or other bacteria.

Rabbit No. 2. March 21, 1900.

Received .5 c.cm. of a suspension of a culture grown on beef-tea injected into large vein of ear. The culture apparently did not contain any spores. The animal died very much emaciated, May 17, 1900.

Many submiliary white nodules in lungs, kidneys, spleen. Near the free edge of the liver there are bright yellow, irregular, badly outlined spots with hyperæmic margins. Fresh teased specimens show protozoon-like bodies in all nodules; none were found in the discolored areas in the liver.

In some of the nodules two small forms are closely approximated and form figure-8 forms. In none of the specimens were any mycelia seen.

Microscopical Examination.—Heart.—A few subendocardial areas in which

there is a slight thickening of the connective tissue with atrophy of some of the muscle fibers. No parasites.

Intestine normal.

Spleen (Fig. 18).—Conglomerate submiliary nodules, consisting largely of epithelioid cells with many giant cells. In the centers of some of them there are small collections of polymorphonuclear leucocytes, some of which show karyorrhexis and beginning disintegration of the protoplasm. They are imbedded in a stringy material which, by van Gieson's method, stains dark brown, with eosin bright red (fibrin). In the peripheral parts of the nodules are many lymphocytes (mostly plasma cells). In the periphery of nodules connective-tissue fibers are found between epithelioid cells.

Parasites.—Moderate number. Mostly in giant cells, adult forms; many collapsed, degenerating forms with little protoplasm or none at all. No bacteria.

Adrenal.—Normal.

Kidney.—Nodules very much like nodules in spleen; perhaps more lymphocytes throughout nodules; marked evidence of formation of fibrous tissue in periphery.

Parasites.—None found in sections, but a few in each nodule in fresh teased specimens. No bacteria.

Liver (Fig. 15).—Extensive necroses of spherical or irregular form (yellow spots). The spaces between the necrotic liver cells are filled with very irregular epithelioid cells. In the central portions there are many polymorphonuclear leucocytes in varying stages of disintegration and much nuclear debris. The necrotic liver cells stain intensely red with eosin. With van Gieson's method the peripheral ones stain light brown very much more like normal liver cells; the central more degenerated ones more darkly. In the periphery of the nodules a few lymphocytes (mostly plasma cells).

Parasites found neither in sections, nor in fresh teased specimens; no bacteria.

In other parts of the liver there are submiliary nodules consisting of epithelioid cells (Fig. 19).

Lung.—Single or conglomerate nodules where the septa are thickened and air-spaces are filled with cells mostly of the epithelioid type. In peripheral parts of nodules one finds connective-tissue fibers between the epithelioid cells. With the epithelioid cells there are found lymphocytes (mostly plasma cells) especially in the periphery of the nodules. In the central portions of some of the larger nodules there are accumulations of polymorphonuclear leucocytes surrounded by necrotic material which stains very intensely with eosin and dark brown with van Gieson's method. In one place a microscopic cavity has been formed by the destructive process. The septa of the adjoining air-spaces are slightly thickened, the air-spaces themselves are collapsed and either partly or entirely filled with large desquamated epithelial cells.

Parasites are present in considerable numbers, mostly young or adult forms. Many exhibit signs of degeneration. They are folded, contain little or no protoplasm, or show vacuolization. In one place are a few sporulating forms surrounded by polymorphonuclear leucocytes. All other forms are enclosed in giant cells.

Uterus and *cerebrum* normal.

Rabbit No. 3, large male. March 21, 1900.

Received .2 c.cm. of a suspension of a third generation of the mould (mycelium with few spores) injected into large vein of ear.

August 2. The animal shows complete paralysis of posterior part of body. Killed.

Post-mortem Findings.—Peritoneal cavity.—No abnormal contents. *Bladder* very much distended, reaches almost to lower surface of liver. Numerous white slightly retracted scars of the size of a pin's head in the *spleen* and kidneys. A few white nodules of the same size in posterior part of *liver*. Larger white hard nodule of the size of a millet-seed on the lower surface of the right half of the *diaphragm*. Just visible greyish-white nodules in both *lungs*. *Heart* normal. In the serosa over the large intestine is a miliary white, hard, projecting nodule. *Brain.*—After removal of skin covering the head, the bone is found to project about 2 mm. above the normal level on both sides over the frontal lobes. The projection is more marked on the left side. The projecting area measures about 3 mm. in diameter and is of a yellowish color with red margin. Underneath it there is an abscess filled with thick semi-fluid pus. On the lower surface the abscess projects into the skull and has caused considerable compression of the left frontal lobe. Brain and meninges otherwise normal. The lower part of the lumbar cord is compressed by a tumefaction and purulent infiltration of the posterior parts of three of the upper lumbar vertebrae and the adjoining tissue.

Examination of fresh specimens.—In the abscess over the left frontal lobe there are a moderate number of parasites; adult forms, some empty shells, three sporulating forms. Otherwise the abscess contains mostly fatty detritus. Similar findings are noted in specimens from the diseased area in the lumbar region, but the specimens contain less fat.

In the nodule in the *lung*, a few adult forms are seen; there is much fat in central part of nodule.

The nodule in the liver is calcified; there are no parasites.

The nodule in the kidney shows one empty shell.

The nodule in the spleen shows several adult and young forms. One parasite shows a figure-8 form. In one spot about eight young forms are enclosed in a common capsule.

The diaphragmatic nodule is filled with pus, which contains many adult and young forms, two sporulating forms, one group of young forms in a common capsule, and several parasites with figure-8 form.

Microscopic Examination.—Spleen.—The capsule shows a slight wrinkling. At the bottom of the depressions there are small masses of fibrous tissue, which differ from the connective tissue of the capsule by an absence of involuntary muscle-fibers, and also by an irregular infiltration with lymphocytes. No *parasites* found in sections.

Liver.—In most places there is a slight (in some a marked) proliferation and moderate lymphocytic infiltration of the periportal connective tissue. Some of the fibroblasts have entered the spaces between the rows of liver cells and are beginning to encroach upon the latter. No nodules; no parasites.

Kidney (Fig. 21).—In the cortex and medulla are seen irregular small areas in which the uriniferous tubules are small. The epithelium in them is

small, not differentiated. Between the collapsed tubules there are strands of fibrous tissue. The glomeruli in these areas show a fibrous thickening of the capsule; in others is seen typical hyalin degeneration. Quite a number of the uriniferous tubules inside and near such areas contain large hyalin casts. The lymphatics in the medullary part of the kidney are very much distended and filled with hyalin material (coagulated lymph?). No *parasites* in sections.

Lung.—The nodules of the lung appear in two different forms. In one the center of the nodule consists of irregularly arranged large cells with a large spongy protoplasmic body. With such cells one finds irregular groups of lymphocytes. The periphery is made up of dence fibrous tissue. These nodules contain a few adult intact or degenerated *parasites.* The other nodules consist of fibrous tissue in which the remnants of the air-spaces appear as gland-like formations with cuboid epithelial cells on their wall. No *parasites* found in such nodules. One of the larger branches of the pulmonary artery is completely obstructed by a thrombus. The thrombus consists of conglutinated blood platelets, red blood corpuscles, some fibrin and in the middle of it there is considerable nuclear debris. There is no evidence of organization. No *parasites.*

Intestine.—The nodule situated in the subserosa has destroyed the muscularis but does not reach into the mucous membrane. The periphery of the nodule is formed by dense fibrous tissue with slight lymphocytic infiltration. In the middle of the nodule there is an irregular necrotic area which is composed of granular albuminous detritus, and nuclear debris in large quantity. There are also a few polymorphonuclear leucocytes and a considerable number of very large irregular cells with large clear nuclei, the protoplasm of which contains remnants of cells and nuclear debris. Interposed between the peripheral and central parts of the nodule one finds a thin layer of granulation tissue composed of large epithelioid cells, lymphocytes, a few polymorphonuclear leucocytes, and some large giant cells. *Parasites.*—Many adult forms are seen in the granulation tissue, mostly in giant cells. In the broken-down centers of the nodules are some adult forms, many empty shells, several sporulating forms. In one spot is seen a shell with about six young forms inside it.

Spinal column.—In the bony parts laterally and posteriorally the medullary tissue is replaced by granulation tissue; many of the remaining bony spicula show evidence of beginning absorption (Howship's lacunae with osteoclasts). In the granulation tissue there are numerous submiliary abscesses, that are either filled almost entirely with polymorphonuclear leucocytes or show polymorphonuclear leucocytes in the periphery and in the center a necrotic mass that consists of albuminous detritus, remnants of cells and nuclear debris. In spots the granulation tissue around these abscesses shows extensive necroses. These necrotic areas contain less nuclear debris. The granulation tissue is in most places surrounded by a layer of cicatricial tissue. There is very little, if any, new formation of blood-vessels. There are comparatively few giant cells in the granulation tissue.

Parasites.—In the granulation tissue are many adult forms, some in giant cells. In many of the abscesses sporulating forms are seen. The spores are

somewhat different in form. In the middle of the mass of spores one usually finds S-shaped forms. Laterally they are mostly biconcave disks or biconcave plates, some three-cornered, other hexagonal. There are also pyramidal forms with concavities on all their surfaces. Of all forms only the peripheral ones stain at all; quite well with hematoxylon or other nuclear dyes, whereas eosin and other protoplasmic dyes stain them less distinctly.

Skull (Fig. 24).—Lesions of the same character, by which in one place the bone is destroyed in its entirety from dura to outer periosteum.

Rabbit No. 4. September 10, 1900.

Small white rabbit inoculated with 1 c.cm. of a milky suspension of culture, containing many spores, into ear-vein.

September 12. A small abscess has formed at the point of injection.

Microscopic examination of the pus shows many polymorphonuclear leucocytes, many spores. A few of the spores have germinated and developed into short hyphae (Fig. 28).

September 13. Hyphae disappearing. Many of the spores are developing into protozoon-like bodies (Fig. 29). The development takes place by simple enlargement. Either the spore grows as a whole or there is first a bulging out on one side with thinning of the spore-membrane at that point. The spore membrane, however, develops directly into the membrane of the protozoon-like bodies.

September 14. Further development of protozoon-like bodies. One finds already several sporulating forms. In one place there is an empty shell. This shell and several more of the later stages are distinctly prickly on the surface (fresh specimen without addition of any chemical). One form has a figure-8 shape. One body is not spherical, but has a decided pear-shape.

September 15. Very many sporulating forms; very many forms with prickly surface.

September 17. Hardly any mould-spores left. Many empty shells. Many of the adult forms show a large central or slighly eccentric vacuole (Fig. 30); one of them has a distinct pedicle (Fig. 30).

September 20. Some pus, which at this time does not contain any of the original spores from the mould, is suspended in a drop of beef-tea, and incubated for 24 hours. After that time numerous hyphae have developed from adult forms (Figs. 32 and 33) and also from spores of protozoon-like bodies (Fig. 34). The hyphae are especially numerous around sporulating forms.

The animal died in the night from October 15 to 16, 1900.

Post-mortem examination, October 16, 9 A. M.

The animal is very much emaciated. At the *site of inoculation* there is a swelling, which extends about 2 cm. from the basis of the ear towards the distal end following the marginal vein. The swollen parts are covered with thick scabs, underneath which there are irregular ulcerations in the skin. The swollen tissues are irregularly infiltrated with pus.

Lungs.—Bright red. Very many, just visible, grey nodules are seen.

Spleen.—Slightly enlarged. A few just visible nodules are recognized.

Kidney.—A few just visible subcapsular nodules. Many larger ones (about the size of millet-seeds) with grey periphery and yellow center projecting over cut surface at the border line between cortex and medulla.

Liver.—No macroscopic lesions.

Bladder.—Much distended.

A few drops of slightly bloody fluid in the peritoneal cavity.

No lesions in the central nervous system.

Experiments on *cold-blooded* animals have been confined to two *frogs*. One was inoculated subcutaneously, the other into the peritoneal cavity. In both experiments the result was absolutely negative.

Collodion sac experiment.—August 25, 1900, a collodion sac containing a little beef-tea and some material from a pure culture with many spores was introduced into the abdominal cavity of a rabbit. August 30. Rabbit killed; some suppuration around sac. Contents consist entirely of spores; no protozoon-like bodies.

EXPLANATION OF PLATES.

(*Figs.* 1–24 *inclusive are microphotographs.*)

PLATE XXXIV.

Fig. 1. × 90. Tubercle-like nodule in liver of first human case.

Fig. 2. × 90. Necroses and abscess-formation in lungs of first human case. In center one sporulating form.

Fig. 3. × 50. Showing general distribution of diseased process in lungs of first human case.

Fig. 4. × 90. Lungs of first human case. Air-spaces filled with granulation tissue consisting of epithelioid cells and lymphocytes. In the center are adult parasites enclosed in a giant cell.

Fig. 5. × 90. Two abscesses containing sporulating forms in the kidney of first human case.

Fig. 6. × 90. Tubercle-like nodule around a glomerulus in the same kidney.

PLATE XXXV.

Fig. 7. × 90. Tubercle-like nodule in pericardium of second human case. In center large giant cell containing adult forms of parasite.

Fig. 8. × 90. Small abscess containing sporulating forms in spleen of first human case.

Fig. 9. × 40. General view of lesions in meninges of second human case.

Fig. 10. × 90. Lesions in meninges of second human case. Higher power. In center small abscess with empty spore shell.

Fig. 11. × 90. Tubercle-like nodule with giant cell and central caseation. Many young and adult parasites. Testis of Guinea-pig No. 2.

Fig. 12. × 17. General view of lesions of testis of Guinea-pig No. 3. Tubercle-like nodules and irregular abcesses, both imbedded in cicatricial tissue.

PLATE XXXVI.

Fig. 13. × 340. Testis of Guinea-pig No. 3, showing parasite in beginning of sporulation and empty spore-shell full of polymorphonuclear leucocytes.

Fig. 14. × 90. Testis of Guinea-pig No. 3. Small abscess containing sporulating forms.

Fig. 15. × 90. Liver of Rabbit No. 2. Extensive necrosis with much nuclear debris and some polymorphonuclear leucocytes in periphery.

Fig. 16. × 340. Testis of Guinea-pig No. 3, showing sporulating form that has recently burst. A polymorphonuclear leucocyte has just entered through rent in capsule.

Fig. 17. × 340. The same. Showing granulation tissue with giant cells and adult forms of parasite. One parasite shows folding of capsule.

Fig. 18. × 90. Spleen of Rabbit No. 2.

PLATE XXXVII.

Fig. 19. × 90. Another part of liver of Rabbit No. 2. Tubercle-like nodule in periportal connective tissue. Very early stage.

Fig. 20. × 90. Lung of Rabbit No. 2. Formation of large giant cells by fusion of desquamated epithelial cells. In one of them an adult parasite.

Fig. 21. × 90. Healed lesion in kidney of Rabbit No. 3.

Fig. 22. × 90. Kidney of Rabbit No. 2.

Fig. 23. × 90. Diaphragm of Guinea-pig No. 2, showing plasma cells on left side; many adult forms; encapsulated sporulating forms in tubercle-like nodule (has been slightly retouched in order to show the sporulating parasite better).

Fig. 24. × 10. General view of lesion of skull in Rabbit No. 3.

PLATE XXXVIII.

Fig. 25. Sporulation of pathogenic mould in potato culture, kept at room temperature twenty days. Spores about 8 μ in largest diameter.

Fig. 26. Gilchrist's mould. Sporulating (?) forms. Potato culture kept at room temperature for twenty days. Bodies vary from 5–9 μ in diameter.

Fig. 27. The same.

Fig. 28. Germination of spores of mould in abscess of ear of Rabbit No. 4, 2nd day.

Fig. 29. Development of protozoon-like bodies from spores of mould in abscess of ear of Rabbit No. 4, 3rd day.

Fig. 30. Unusual form of parasite found in abscess of ear of Rabbit No. 4, 5th day.

Fig. 31. Germination of spores of mould when transferred to new medium (gelatine drop, kept at room temperature for 48 hours). The spore membrane always bursts at the side.

Figs. 32 and 33. Development of mycelium from protozoon-like bodies when kept in artificial media (hanging drop of beef-tea kept for 24 hours in incubator).

Fig. 34. Development of mycelium from spores of protozoon-like bodies.

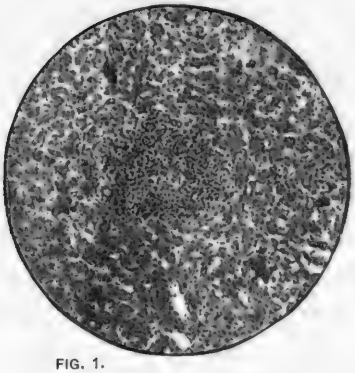

FIG. 1.

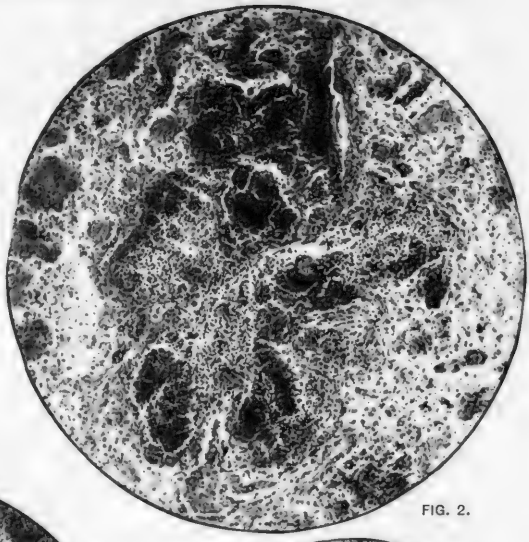

FIG. 2.

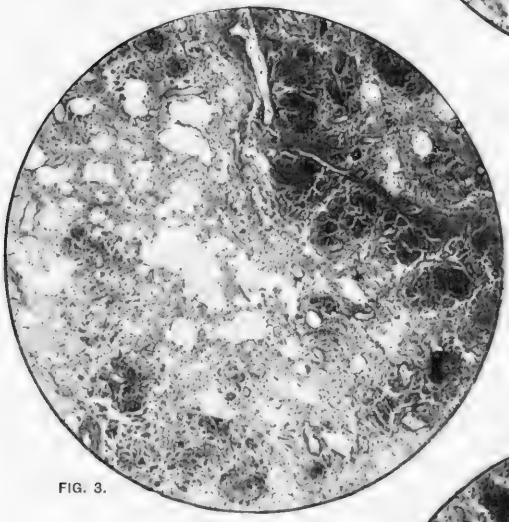

FIG. 3.

FIG. 4.

FIG. 5.

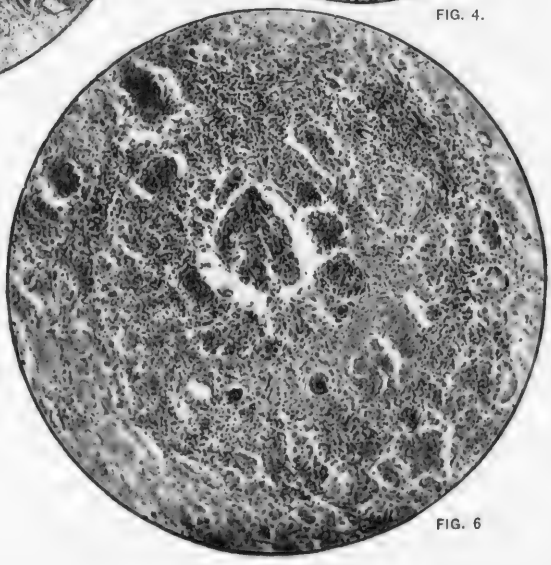

FIG. 6

FIG. 7.

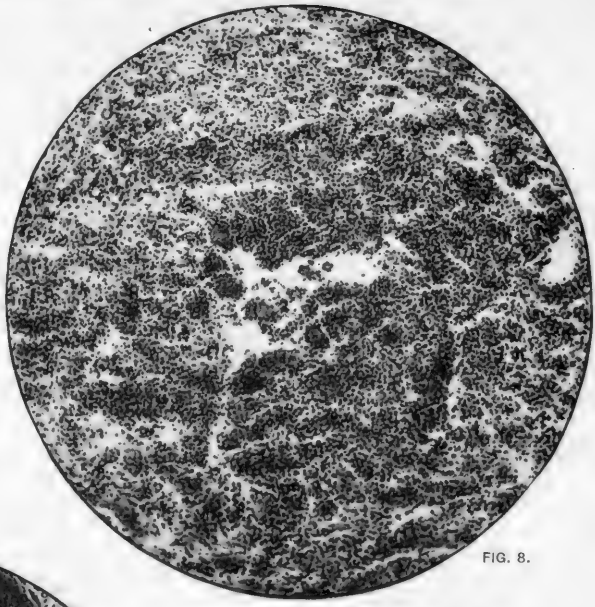

FIG. 8.

FIG. 9.

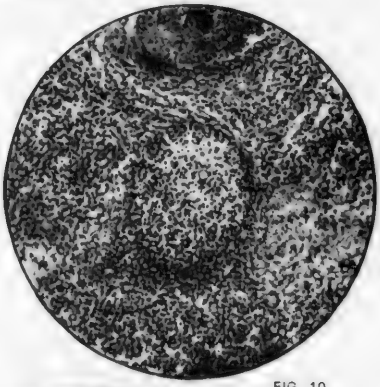

FIG. 10.

FIG. 11.

FIG. 12

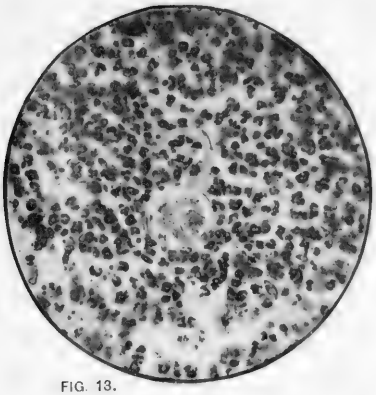

FIG. 13.

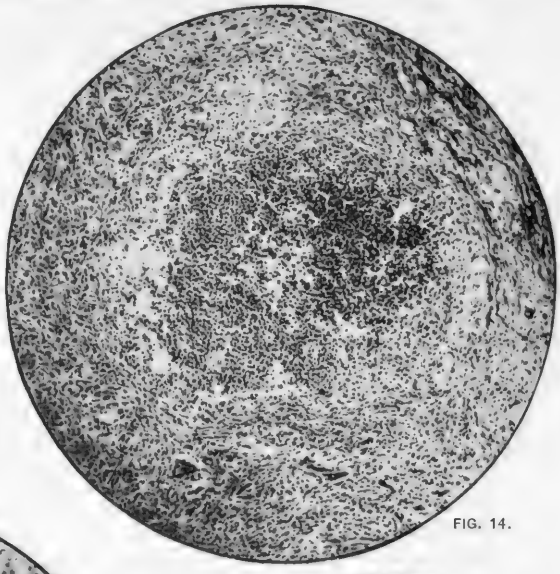

FIG. 14.

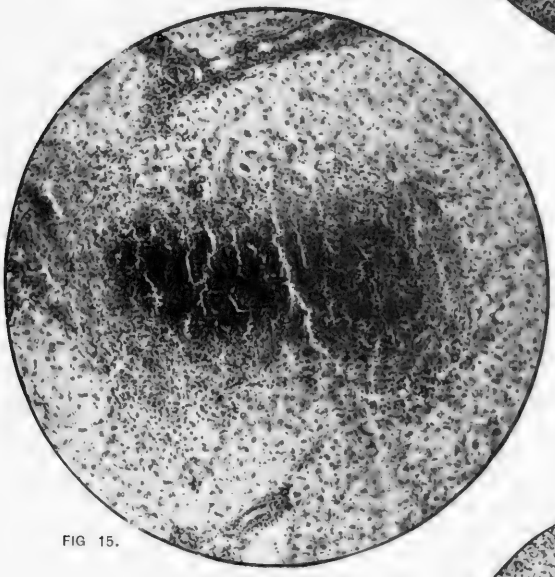

FIG. 15.

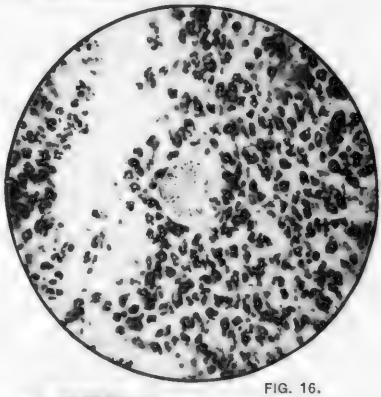

FIG. 16.

FIG. 17.

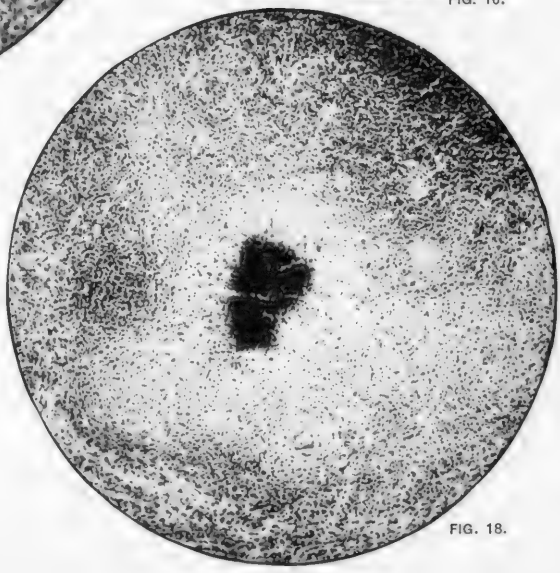

FIG. 18.

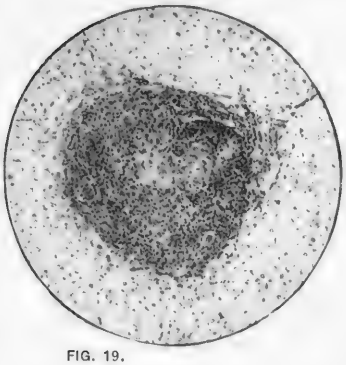

FIG. 19.

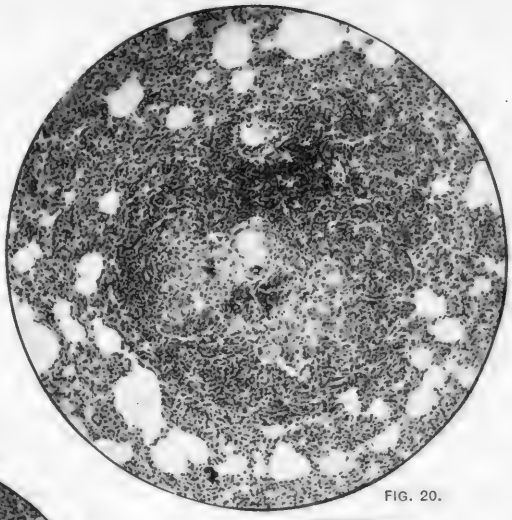

FIG. 20.

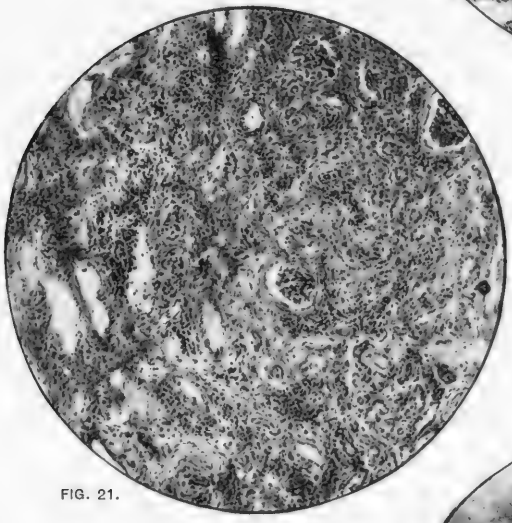

FIG. 21.

FIG. 22.

FIG. 23.

FIG. 24.

FIG. 25.

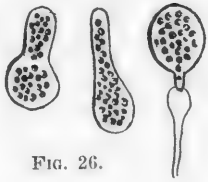

FIG. 26.

FIG. 27

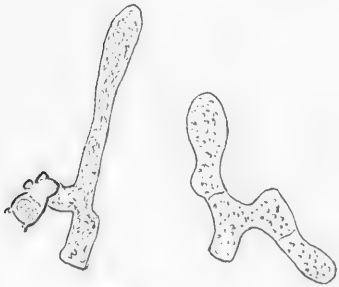

FIG. 28.

FIG. 31.

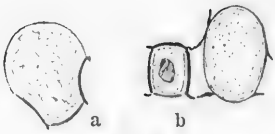

FIG. 29.

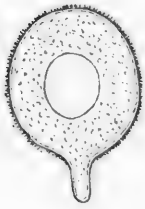

FIG. 30.

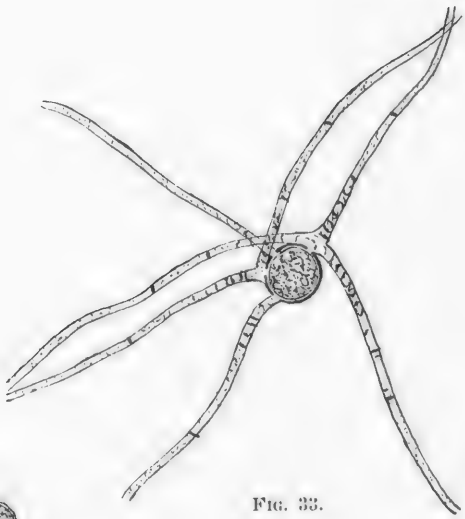

FIG. 33.

FIG. 34.

FIG. 32.

THE TOXIC EFFECTS OF FORMALDEHYDE AND FORMALIN.

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(From the Pathological Laboratory of Rush Medical College, Chicago.)

Although the germicidal effects of formaldehyde and formalin have been in the last few years the subject of much discussion, yet the study of the action of these substances upon the animal economy has been practically neglected. Much of the literature on this subject is of little value because supported by insufficient experimental evidence, and because overhasty conclusions have been drawn. In the following pages I have collected the literature which bears most directly upon the subject of my paper. Wherever the term *formaldehyde* is used in this article, the gas formic aldehyde, CH_2O, is referred to. By the term *formalin* is meant a 40% solution of formaldehyde in water. The percentage of solutions relates to the amount of *formalin* in the solution. Thus a 10% formalin solution contains 4% of formaldehyde.

In the experiments, immediately after the death of an animal, brought about naturally, or induced by chloroform, ether or a blow on the head, the tissues were fixed in Zenker's fluid; in a few instances alcohol was used. In staining, eosin and haematoxylin were employed, supplemented when necessary by staining for fat with Sudan III, by Weigert's fibrin stain, and by polychrome methylene blue. When no special stain is designated, the description refers to the picture shown by eosin and haematoxylin.

The fixing action of formalin itself is to be remembered; and also that in consequence, a cell killed by contact with the chemical may histologically show no evidences of death.

I. CHANGES IN THE LUNGS AFTER INHALATION OF FORMALDEHYDE.

The following experiments were performed in a room of 5.5 cubic metres capacity. A large door opening into the room was closed but

no attempt was made to prevent the egress of the formaldehyde or the ingress of air through the keyhole, cracks in the door, etc. Formaldehyde gas was generated by slowly volatilizing paraformaldehyde pastils in a Schering disinfecting lamp. The lamp was set in the middle of the room, the animals being in a cage near the door. In all, nine animals were used.

Exp. 1.—A guinea pig and a rat were exposed for 1½ hours; 3 grm. paraformaldehyde volatilized.

Exp. 2.—A guinea pig and a rat were exposed for 3½ hours to an atmosphere in which 5 grm. of paraformaldehyde had been volatilized.

Exp. 3.—A guinea pig and two rats were exposed for 6 hours to an atmosphere in which 11 grm. of paraformaldehyde were slowly volatilized.

When removed, the animals were slightly dazed but otherwise apparently well. At post-mortem, forty-two hours later, the lungs of the animals were somewhat reddened. In the third experiment crepitation was slightly diminished in the lungs of all three animals. The bronchi contained a small amount of mucus.

Cultures were made from the nose, mouth, throat and lungs of each of the animals. In three of the animals micrococcus tetragenus was isolated from the mouth. A single colony of the same bacterium was obtained from the lungs of one of the guinea pigs. All the other cultures were sterile.

Histological examination reveals distinct inflammatory changes in the lungs of the animals. The lungs of the rat and guinea pig exposed for one and a half hours show marked congestion. Many of the alveoli are filled with a homogeneous red-staining exudate. Slight desquamation of the alveolar lining cells has occurred. Many polymorphonuclear leucocytes are found in the capillaries, in the connective tissue of the lung and free in the alveoli. The eosinophiles are numerous (Fig. 1). Mononuclear leucocytes are present but are few in number. The bronchi are filled with serum containing a few leucocytes and desquamated and degenerated bronchial epithelium. The deeper layer of the cells of the bronchi are still

attached to the basement membrane. In the peribronchial connective tissue many leucocytes are found. Numerous red blood corpuscles are mixed with the leucocytes in the alveoli and bronchi. The trachea shows no change.

In the two animals exposed for three and a half hours, the changes are more severe. The most striking difference is noted in the character of the leucocytic infiltration. Localized areas of pneumonia are found involving either a single alveolus or several alveoli; often these are peribronchial or perivascular (Fig. 2). The major part of the leucocytes which have accumulated are of the mononuclear variety. The polymorphous variety has also increased but not in proportion to the mononuclear variety. The number of leucocytes and alveolar epithelial cells in the bronchi is larger than in the foregoing experiment.

In the three animals exposed for six hours, the pneumonia is similar to that just described, but the process is more diffuse and involves large areas of the lung substance. A beginning tracheitis is found in the animals exposed for three and a half hours (Fig. 3), but in the last series of experiments this is more marked. The blood-vessels of the submucosa are congested and leucocytic infiltration has occurred. The latter is mainly eosinophilic. Many leucocytes are found between the epithelial cells lining the trachea. The epithelial cells themselves show slight degenerative changes, and are often desquamated.

Exp. 4.—A spaniel, wt. 25 kilo., was left for 5 hours in the before described room. The formaldehyde lamp was set on a high shelf and 5 grm. of paraformaldehyde volatilized in the course of the first half hour. The door of the room was opened several times. The slight dry cough present when the dog was removed from the room became moist in character several hours later. On the following day the animal coughed incessantly; and this coughing continued until the third day when the dog was bled to death.

Exp. 5.—A large cat was allowed to run about in the room while 250 cc. of methyl alcohol were oxidized to formaldehyde in a Moffat lamp. Time, 2¾ hours.

At autopsy the lungs are less crepitant than normally, œdematous, and float low in water. Small areas of consolidation are scattered through the lung substance. The bronchi are filled with a greyish, creamy mucus. The trachea is covered with mucus.

By histological examination a diffuse pneumonia, with here and there areas of more intense infiltration, is found to be present in the lungs of both animals. There is general vascular congestion. In the dog the blood-vessels contain an enormous number of eosinophiles and other polynuclear leucocytes. Only rarely can a mononuclear leucocyte be found. In the cat the polymorphonuclear leucocytes are much less in evidence, and the mononuclear forms are predominant. Many of the alveoli are filled with a homogeneous red-stained exudate. This is most marked in the cat in which over half of the alveoli are filled. The leucocytes are mainly of the polymorphous variety, and are most numerous in the walls of the alveoli. The alveoli themselves contain leucocytes and large epithelioid cells which probably represent desquamated alveolar epithelium. The bronchi are filled with large numbers of leucocytes, broken down bronchial epithelium, and granular detritus. The superficial lining cells of the bronchi are swollen and stain poorly; the cellular reticulum is loosened and the cells have lost their sharp contour. The nuclei are swollen and stain palely. Between the cells are found many leucocytes. Desquamation is common, and, when severe, the submucosa is entirely uncovered. In the submucosa and in the peribronchial connective tissue large numbers of leucocytes are found. A more severe bronchitis is found in the dog than in the cat.

It can at once be seen that my observations are directly opposed to those of Aronson, Phuhl, Rosenberg, Fairbanks, Grawitz, Babes, de Schweinitz, Kobert and Moeller, all of whom conclude that formaldehyde produces no effects upon the animal economy.

Aronson (1) subjected several white mice and a rabbit to the vapors of formaldehyde and found, after twenty-four hours, that the animals were alive and well. A histological examination showed no tissue changes. Fairbanks (2) found the same true of rabbits and mice

which he had subjected to formaldehyde inhalation for twenty-five hours. De Schweinitz (3) kept a calf in a 2% atmosphere of formaldehyde for five hours, and beyond a slight watering of the eyes and an occasional cough, could find no deleterious effects. Kobert (4) found that guinea pigs permitted to run loose in a room during formaldehyde disinfection did not become sick. Moeller (5) allowed six guinea pigs to run about in rooms which were disinfected upon four consecutive days. After that time he found the animals lively and well and they remained perfectly well thereafter. Doty (6) could find no tissue changes in insects, fowls, guinea pigs, mice, etc., subjected to formaldehyde gas for periods of from three to fifteen hours; occasionally a guinea pig would show a slight inflammation of the respiratory tract.

Klipstein (7) allowed two rabbits to inhale formaldehyde gas from a heated formalin solution. The first of these inhaled the gas for one-half hour; the other for one-half hour on three consecutive days. The post-mortem examination showed rhinitis, laryngitis, tracheitis and catarrhal bronchitis. These changes were more severe in the second animal. A muco-purulent material was found in the bronchi. The lungs were normal. Except for the last statement these findings agree with those in my experiments.

Harrington (8) has found that cats and dogs accidently confined in rooms undergoing disinfection rarely survive the process. As proof he cites two experiments of his own, and refers to the experience of several persons daily engaged in house disinfection. Likewise, Francis, of the health department of St. Louis, Mo., several times put rats into rooms undergoing disinfection. Except in one instance they were all killed in one and a half hours. Two guinea pigs, likewise experimented upon, remained well. The death of the investigator Staub has been attributed to the inhalation of formaldehyde.

Schlossman has worked with a combination of glycerine and formalin (glyco-formol) and urges the necessity of wearing a gas mask when entering a room in which disinfection has been performed.

II. INJECTION OF FORMALIN INTO THE STOMACH.

Formalin was injected directly into the stomach by means of a soft rubber catheter introduced through the oesophagus. The symptoms following the injection of formalin differed in the different animals. In all, eleven animals were used.

Exp. 1.—Rabbit, wt. 500 grm. Injected 8 cc. of concentrated formalin. The animal falls upon its side and expires in five minutes.

Exp. 2.—Rabbit, wt. 500 grm. Injected 4 cc. of 10% formalin. The animal died immediately.

Exp. 3.—Rabbit, wt. 510 grm. 6 cc. of 10% formalin were injected. The animal died in one minute.

Exp. 4.—Rabbit, same wt., size and age as preceding. Injected 6 cc. of 10% formalin. The rabbit loses weight rapidly and dies in three and a half days.

Exp. 5.—Kitten, wt. 902 grm. Injected 5 cc. of 10% formalin. Catheter was bitten in half on withdrawal, and a few drops of the formalin got into the mouth. Death ensued in one hour.

Exp. 6.—Kitten, same wt., size and age as preceding. Injected 1 cc. of 10% formalin. On the following day the kitten has developed an intensely foul breath which continues to the day of death, three and a half days later.

Exp. 7.—Kitten, wt. 1021 grm. Injected 3.5 cc. of 10% formalin. Death occurs on the ninth day after the injection of the formalin.

Exp. 8.—Rabbit, wt. 1380 grm. Injected 8 cc. of 5% formalin. Death occurs in six days.

Exp. 9.—Terrier, wt. 53 kilo. Injected 17 cc. of 5% formalin. After a minute the dog vomited a frothy mucus which had a strong odor of formalin. After half an hour the animal becomes quiet. Death occurs in forty hours.

Exp. 10.—Kitten, wt. 980 grm. Injected 3 cc. of 10% formalin. The kitten is killed three weeks after the operation, in which time it has grown and gained in weight.

Exp. 11.—Rabbit, wt. 1401 grm. Injected 6 cc. of 5% formalin. The animal ate and increased in weight. After a month it was killed.

On post-mortem examination no macroscopic changes except a reddening of the mucous membrane of the stomach and duodenum are

found in the animals that died immediately after the injection of the formalin. Histologically, only an intense vascular congestion exists.

The stomach of the rabbit in the fourth experiment contains only a few drops of a turbid liquid. The mucous membrane is grayish, soft, and can be easily scraped off; the pyloric end of the stomach is ulcerated. The intestinal tract is empty, except the caecum, which is filled with a soft, brownish, gruel-like material.

Histologically, the serosa and the muscularis show no change. The submucosa has undergone loosening so that the connective tissue fibrillæ are widely separated from each other. The blood-vessels are markedly congested, but no haemorrhages have occurred. There is only a slight leucocytic infiltration. An increase in the staining reaction of the gland cells is the most striking change noted in the mucosa; all the cells are more deeply colored with haematoxylin than normally, and in the late stages of degeneration they stain a uniform, deep purple. The changes are more severe upon the rugæ than in the tissue between them. In the latter locality the cells are swollen and granular. The outlines of the cells are lost and the nuclei are irregular in shape. The peptic cells are swollen, granular, and stain red with eosin. Upon the rugæ the glandular cells are contracted. Many of the nuclei are lost, while those remaining are misshapen beyond recognition. Desquamation of the gland cells is everywhere present. At the top of the rugæ desquamation of all these cells has occurred, so that the gland cells are represented by a deep purplish-staining detritus within the connective tissue casing of the tubules. Many of the tubules are widely distended with necrotic matter. In some places only the intertubular connective tissue is found remaining. Areas are found in which a total loss of the mucosa has taken place, exposing the bare submucosa beneath. The large ulcer seen with the naked eye is found to be due to such a loss of the mucosa.

The stomach of the dog in Exp. 9 contained a few cubic centimeters of a turbid fluid similar to that found in the stomach of the rabbit just described. The mucous membrane was similarly gray and soft.

The histological findings are similar to those previously described, but the more deeply stained glandular cells extend only half way down the tubules. The cells are separated from each other, and a well-marked desquamation is present. A marked infiltration with polymorphonuclear and large and small mononuclear leucocytes has occurred. This is confined to the mucosa and submucosa. The changes, gradually diminishing in severity, extend through the duodenum into the small intestine for the distance of about a foot.

The kitten in Exp. 5, which died an hour after the injection of the formalin, showed the following changes on post-mortem examination. The mouth, pharynx and posterior nares are red and covered with mucus. The mucous membrane of the oesophagus is reddish in color and soft; and can be scraped off leaving a raw surface studded with red points. The stomach is filled with food from which emanates a strong odor of formalin. The mucous membrane is soft and reddish. All the vessels are markedly congested. Similar changes are found in the duodenum.

Histological examination shows a marked vascular congestion of the tissues of the mouth, pharynx and oesophagus. The connective tissue elements of the oesophagus are loosened so that the fibrillæ are widely separated from each other. No changes can be found in the epithelial cells. The congestion of the stomach and duodenum is very marked; in the latter location the separate capillaries can be traced to the tip of the villi. The glandular elements show no change. An acute oedema, manifesting itself by exudation into the submucosa, has occurred and in consequence the stomach wall is the seat of an immense bleb. The exudate makes up about half the thickness of the wall, and is stained homogeneously red. There is no leucocytosis.

In the stomach of the kitten in Exp. 6 are found 4 cc. of a dirty, turbid fluid. The mucous membrane is grayish, soft and can be scraped off easily. The stomach of the kitten in Exp. 7 is filled with a soft, pus-like material. The mucous membrane is grayish and soft; in some parts it has ulcerated. The mucous membrane of the

duodenum has a similar character. The gall-bladder is enormously distended with bile, and a bristle can with difficulty be pushed through the bile duct.

The microscopic findings in these two experiments are quite similar. In both cases we have to do with an intense gastritis. In the first of them the leucocytosis is most severe in the mucosa; the submucosa is also infiltrated with leucocytes, but in the muscularis only a few are seen. In Exp. 7 the inflammatory process involves the muscular tissue especially in those parts where the mucous membrane has ulcerated. The leucocytes present are mainly of the mononuclear variety; only a few polynuclear leucocytes are seen. The gland cells of the superficial parts of the mucosa are in various stages of disintegration. The infiltrating leucocytes show karyolysis and karyorrhexis. A granular, violet stained, necrotic material, mixed with mucus and broken down nuclei is found lying upon the mucosa. The mucosa and submucosa of the duodenum in Exp. 7 is the seat of marked inflammatory changes. This probably brought about an atresia of the gall duct, and a consequent distension of the gall-bladder with bile.

By far the most interesting series of pathological changes are found in the rabbit of Exp. 8. The stomach contains about 8 cc. of a foul-smelling, brownish, gruel-like liquid. The mucous membrane is deeply red, mottled with smaller areas of white, and traversed by thick, firm ridges. The whole is covered by mucus mixed with a whitish necrotic material; when the hand is brushed over the lining of the stomach the mucous membrane is scraped off, and a raw surface studded with red points is left.

On histological examination the changes in the stomach wall are found to be irregularly distributed and of varying intensity. Desquamation of the serosa has occurred in some places. The connective tissue of the subserosa retains a distinct bluish tinge after staining with haematoxylin. The muscularis shows changes ranging from a slight inflammatory condition to total necrosis. In those regions in which the muscular coat is least affected the muscle cells are slightly

swollen and stain palely. Many of the muscle cells are entirely separated from their bundles, and are in various stages of degeneration. With these changes is found an infiltration of polynuclear and mononuclear leucocytes. The muscular layer is affected first on the side nearest the mucous membrane, whence the changes progress downward until the serous side of the muscularis is reached.

The submucosa is the seat of an enormous fibrinous exudate which varies in thickness in different parts of the sections; at one time the layer of fibrin is scarcely recognizable, at another it is three times as thick as the normal wall. The ridges mentioned in the anatomical description are entirely due to submucous fibrinous exudation. The exudate, especially in its deeper portions, contains many polymorphonuclear leucocytes; some mononuclear leucocytes are also found. The muscularis mucosæ shows changes similar to those of the muscularis. The mucosa shows a series of changes; in the least affected parts only a few swollen cells are found, but in the more altered parts there is slight desquamation and necrosis accompanied by leucocytic infiltration. The entire thickness of the mucosa is at times wholly destroyed; it is then found lying upon its basement membrane as a necrotic mass, stained in various ways from a light red to a deep purple. Large areas of haemorrhage into the mucosa and underlying fibrinous exudate are found; the blood-vessels are enormously distended and in places the walls of ruptured vessels are seen.

The stomachs of the animals in Exp. 10 and Exp. 11 were filled with food; and no macroscopic or microscopic changes were demonstrable.

The wide range in the character of the pathological changes has forced me to describe these experiments in detail. Briefly summarized, the histological findings in the eleven stomachs examined are as follows:

Intense vascular congestion in Exp. 1, 2 and 3.

Infiltration of the mucosa with leucocytes together with necrosis of gland cells in Exp. 4 and 9. The necrotic material is hyaline and stains deep purple with haematoxylin.

Intense vascular congestion with acute oedema of the submucosa in Exp. 5.

Gastritis involving the entire wall of the stomach, and characterized by marked leucocytosis, karyorhexis, and necrosis in Exp. 6 and 7. The necrotic material is granular and stained violet with haematoxylin.

Marked leucocytosis and necrosis in all the tunics of the stomach, together with an intense fibrinous and haemorrhagic exudation into the submucosa in Exp. 8.

No recognizable changes in Exp. 10 and 11.

It is evident that I failed in my attempt to get a series of experiments which would show a progressive increase in the severity of the pathological changes corresponding with a progressive increase in the strength and amount of formalin injected. Likewise the severity of the symptoms following the injection of formalin into the stomach is by no means proportionate to the strength or amount of the injected chemical; nor is there a definite series of symptoms following the injection of a definite amount of formalin of a definite strength. The results obtained in the first three experiments in this series lead me to class formalin with that rare group of poisons which are capable of producing sudden death when swallowed.

Other things being equal, it might be expected that the rapidity with which death occurs after the injection of formalin into the stomach would be influenced by the following conditions: The amount or strength of the chemical; the time of retention after ingestion (absence of vomiting); the condition of the stomach; whether empty or not and the size of the animal experimented upon.

How small a rôle these factors play may be judged from the following: Although the rabbit of Exp. 2 was heavier and received only two-thirds the amount of formalin that was given to the rabbit of Exp. 4, the first died immediately after the ingestion of the chemical, while the second lived several days. Similarly, although the rabbits in Exp. 3 and 4 were identical in size, weight and age, and were injected under the same conditions, with the same amount of formalin, one died immediately while the other survived several days. In Exp. 6 the injection of 1 cc. of 10% formalin proved fatal in three and a half days. In Exp. 7 the injection of 3.5 cc. of formalin of the same

strength was followed by an attack of vomiting, and the animal lived nine days. It might be concluded that the animal's life was preserved because the toxic agent was vomited. Contrary to this conclusion are Exp. 8 and 9, for while the rabbit in Exp. 8 lived six days after the ingestion of 8 cc. of 5% formalin, the dog in Exp. 9 survived the administration of 17 cc. of the same strength by only forty hours, even though the animal began to vomit almost immediately after the ingestion of the chemical.

That the weight of the animal is not an important factor is evidenced by the fact that the dog, weighing nearly fifty times as much as the rabbit, was unable to withstand a proportionately smaller dose. The presence of food in the stomach does not seem to influence the toxic action of the chemical. In Exp. 1 and 2 post-mortem examination showed the stomachs filled with food yet both died immediately after the injection of the formalin. The rabbit of Exp. 4 was kept for several hours without food. It survived the injection of formalin for several days, yet its fellow, kept under exactly the same conditions, died immediately after the operation. In Exp. 9, on the other hand, the rapid death of the dog might be in part at least attributed to the ready absorption of the chemical by an empty stomach, for the animal had been kept without food for twelve hours.

That small amounts of formalin when taken into the stomach are capable of producing deleterious effects upon the animal economy is evidenced by the experiments of Annett and Grady.

Annett (9) noticed in kittens fed with milk containing formalin, loss of appetite and diarrhoea with noisy, gaseous motions, gaseous distension of the abdomen and roughening of the fur; in some instances there were emaciation and death. The younger animals were most susceptible, and showed, in contradistinction to the control kittens, little increase in weight with increase in age. F. F. Grady, of the health department of Chicago, has obtained similar results.

III. INTRAPERITONEAL INJECTION OF FORMALIN.

In this series of experiments twelve guinea pigs, two rabbits and five dogs were used. The injections were made through the abdominal wall, under antiseptic precautions, by means of a hypodermic syringe.

The symptoms following the injection of formalin into the peritoneum are fairly constant, and vary in intensity according to the strength and the amount of the injected chemical.

The anatomical findings after intraperitoneal formalin injections are quite similar. After the injection of 1-1000 formalin, macroscopic changes cannot be discovered. Sometimes there is slight vascular congestion. Marked vascular congestion with slight exudation follows the injection of 1% formalin. The intestines are usually contracted, especially at the points where they rub against the abdominal wall or against each other. When undiluted formalin is injected an abundant inflammatory exudate accumulates. Two days after the injection of 3.5 cc., I withdrew 160 cc. of a turbid, bloody fluid from the abdominal cavity of a dog. After coagulation the coagulum constituted 70% of its bulk. In the second dog 140 cc. of ascitic fluid were found post-mortem three days after the injection of 2.5 cc.; 90% of the total volume was formed by the clot after coagulation of the fluid. In the abdominal cavity of a third dog 50 cc. of an intensely bloody fluid were found eight days after the injection of 4 cc. of undiluted formalin. Histological examination of the ascitic fluid reveals desquamated epithelium, red blood corpuscles, large numbers of leucocytes and granular detritus.

The intestines after the injection of undiluted solutions are usually contracted. At the points of irritation are found ecchymoses varying in size. When these are of a mild grade they appear as delicate, subserous, reddish-brown or purple lines running parallel with the long axis of the gut. In the severer cases the entire thickness of the intestinal wall is soft and blood-soaked. Those parts of the intestinal tract which are protected by the omentum or by mutual contact may suffer but slightly in the destructive process, but the omentum may suffer severely. Slight ecchymoses are present in several of the ani-

mals even after the use of 1% solutions. When undiluted solutions are employed the fat columns of the omentum are seen as dark red, boggy masses of clotted blood. The parietal peritoneum shows changes similar to those described above. In one of my dogs extravasation of blood was present through the entire thickness of the abdominal wall. Fibrin is commonly found upon the omentum and mesentery, even when only dilute solutions (1-250) have been employed. In one of the dogs the pancreas was embedded in a fibrinous capsule. In another, fibrinous adhesions existed between all the pelvic viscera. The fibrin was always soft and oedematous.

The endothelial cells of the peritoneum after the injection of 1-1000 formalin, swell, show nuclear degeneration, and are loosened from the basement membrane. Sometimes single cells are found lying upon the connective tissue; at other times small plates of endothelial cells are found in process of desquamation. The muscularis is contracted and its nuclei compressed. In the outer muscular sheath the number of nuclei are diminished. Leucocytic infiltration, mainly polynuclear, has taken place into the serosa and between the layers of the muscularis; it is also found well-marked in the connective tissue of the submucosa. The mesentery is similarly infiltrated. Marked vascular congestion prevails.

Solutions of 1-250 formalin produce similar lesions.

All of the above changes, occurring with greater severity, are found after the injection of 1% formalin. The pathological findings range from an intense inflammation to total necrosis. The connective tissue elements stain intensely red with eosin and show a loss of nuclei. The mucosa is often represented by a mass of blue stained, necrotic material. The submucosa is crowded with polynuclear, and large and small mononuclear leucocytes; the mesentery shows similar changes. Marked vascular congestion and extravasation of blood are general. Abundant fibrinous exudation into the mesentery was found in some of the sections.

The following histological changes in the intestine were found after the injection of undiluted formalin into the peritoneal cavities of dogs:

The peritoneal lining cells are entirely lost in some places; in others, a few poorly stained cells still adhere to the subserosa. The subserosa is hyaline and stained red; its nuclei are decreased in number, misshapen and fragmented. The nuclei of the outer muscular tunic are lost and the muscular tissue is bright red, brownish or purple in color. The same is true but to a less marked extent of the inner muscular layer. The cells lying nearest the supporting connective tissue always suffer the most. The submucosa shows a fairly well-marked leucocytic infiltration. The mucosa suffers only slightly. Only in the dog which lived ten days after the injection could leucocytes in any considerable number be found in the subserosa and muscularis. The parietal peritoneum suffers changes similar to those affecting the visceral.

The most striking feature in these cases is the intense haemorrhage; in some of the sections examined this occupies the entire thickness of the intestinal wall except the mucosa. Blood cells or blood pigment are so abundant that the tissues into which the haemorrhage has occurred are unrecognizable. Well-preserved red blood corpuscles and granular pigment are often associated in the same section; and while one part of the intestinal wall shows naught but red cells other parts may show nothing but blood pigment. The mesentery is in all cases the seat of fibrinous inflammation, often accompanied by profuse haemorrhage.

The peri-pancreatic and interlobular connective tissue of the pancreas of the dog before alluded to is the seat of an intense fibrinous inflammation; the fibrin is delicately fibrillar, and mixed with leucocytes and granular necrotic material formed from the neighboring pancreatic parenchyma. The changes in this organ diminish in severity as its more central portions are reached; here the cells are ill contoured, granular, and show evidences of nuclear degeneration.

The peritoneal fat suffers markedly under the action of formalin. Frequently the fat cells are found filled with purplish, hyaline material. At other times, instead of the changes described above,

necrosis of fat cells has occurred and is accompanied by intense leucocytic infiltration. In the mesenteric fat, the deleterious action of the formalin most often manifests itself by the production of a fibrinous inflammation. Leucocytosis, karyolysis and karyorrhexis are often extreme.

The results of an experiment performed by Adler (10) agree well with my own. This investigator introduced several pith balls saturated with 4% formalin into the peritoneum of a rabbit. After twenty-four hours small ecchymoses were found on the surface of the colon where the pith cubes, surrounded by a soft, translucent, mucoid exudate had lodged. Microscopical examination of the cubes revealed polynuclear and large and small mononuclear leucocytes, plasma cells, "mastzellen," red blood corpuscles, tissue cells and epithelioid cells.

Borst (11) introduced fish bladders filled with blood into the peritoneal cavities of rabbits. When the bladders were sterilized in formalin and then washed in water, the usual cellular infiltration did not occur. This he believed to be due to a swelling of the cells in the fish bladder, or to a negative chemiotaxis exerted by the retained formalin.

The pancreatitis in one of my experiments is similar to the pancreatitis associated with fat necrosis which Flexner (12) produced in two dogs by injecting 2% formalin into the pancreatic ducts.

Experiments to determine the lethal dose of formalin introduced intraperitoneally were performed upon guinea pigs with the following result:

(a) Two guinea pigs died within two days after the injection of 1 cc. of a 1-1000 formalin solution for each 100 grm. of body weight.

(b) Five guinea pigs died within twenty-four hours after the injection of 2 cc. of 1-1000 formalin for each 100 grm. of body weight.

(c) One guinea pig, weighing 345 grm., died three days after the injection of 6 cc. of 1-1000 formalin.

(d) One guinea pig, weighing 306 grm., withstood the injection of 3 cc. of 1-1000 formalin; at the end of four weeks the animal weighed

400 grm., and withstood the injection of 8 cc. of formalin of the same strength.

(e) Four guinea pigs were killed by 2 cc. of 1% formalin in periods ranging from six to twelve hours.

IV. INJECTION OF FORMALIN INTO THE LUNG.

In these experiments slow injection through the chest wall was resorted to.

Exp. 1.—Guinea pig, wt. 405 grm. Injected 1 cc. of 1-1000 formalin into right lung.

Exp. 2.—Guinea pig, wt. 372 grm. Injected 2 cc. of 1-1000 formalin.

Both animals remained apparently well. The first was killed on the sixth and the other on the second day after the injection.

On post-mortem examination the lung on the injected side is not as perfectly collapsed as that of the other side, and on cross-section the lung tissue feels slightly granular.

Exp. 3.—Terrier. Injected 5 cc. of 10% formalin into right lung. After two days, during which the animal coughed from time to time, the dog was killed.

Post-mortem shows only the right middle lobe to be affected. This does not collapse, is firm, and does not crepitate; pieces of it sink in water. The cut section is brownish-red and from it drips a light, straw-colored fluid. In the center of the lobe is found a sharply defined red area the size of a dime. The bronchi contain thin mucus.

Exp. 4.—Terrier. Injected 3 cc. undiluted formalin into right lung. Death occurred in 18 hours.

On post-mortem examination the left lung is pink in color and crepitates throughout. The right lung is dark red, firm, non-crepitant, and sinks in water; from the cut section drips a bloody fluid. In the center of the lower lobe is found a cavity 2 cm. in diameter filled with a bloody, necrotic material.

Exp. 5.—Large cat. Injected 3 cc. of undiluted formalin into the right lower lobe. The animal died immediately.

On post-mortem examination a brownish-black solid area, the size of a small cherry, is found in the right lower lobe. The bronchi of the corresponding side contain bloody froth.

Histological examination of the lungs of the animals in the first two experiments reveals pneumonia on the right side. There is a

slight leucocytic infiltration of the alveolar walls, together with capillary congestion. Scattered throughout the lung substance, but especially common about the larger blood-vessels and the bronchi, are circumscribed areas of leucocytic infiltration. Here the alveoli are filled with eosinophiles, mononuclear and polynuclear leucocytes. Sometimes red blood corpuscles are found, but it is possible that their presence is due to the trauma consequent upon the hypodermic puncture. There is desquamation of the lining cells in some of the bronchi.

In Exp. 3 the pneumonia is much more severe. The dark area described in the anatomical findings is made up of red blood corpuscles, blood pigment and necrotic lung tissue. About this necrotic focus is a zone of intense leucocytic infiltration. Leucocytosis of a less severe type extends throughout the tissue of the affected lobe. About the blood-vessels and bronchi denser areas of infiltration are noted. The leucocytes are mainly of the polynuclear variety. Eosinophile and mononuclear leucocytes are comparatively rare. All the alveoli are filled with a homogeneous, red-stained exudate, mixed with leucocytes, strands of fibrin and desquamated alveolar epithelial cells. There is intense capillary congestion and the large vessels are also well filled. Extravasation of blood has occurred in many localities. The bronchi contain leucocytes, red blood corpuscles and necrotic material. Total loss of bronchial epithelium is a common feature of the bronchitis. The walls of the bronchi are oedematous and infiltrated with leucocytes.

The lungs in Exp. 4 show practically the same characteristics as those in the experiment just described. There is greater extravasation of blood and in places, actual breaks in the vessel walls can be made out.

In Exp. 5 the only discoverable change is a most intense capillary congestion. Except for an occasional red blood corpuscle the alveoli are empty.

V. SUBCUTANEOUS INJECTION OF FORMALIN.

Formalin, varying in strength from 0.1% to 10%, was injected under the skin of the hind leg, or under the skin over the ribs of guinea pigs and rabbits.

The histological findings were, in all cases, quite similar. They varied in severity and extent, with the strength and with the amount of injected formalin. The characteristic lesion is an intense exudation into the subcutaneous tissues. There is found underlying the skin a yellowish, jelly-like mass, from which when cut across drips a large amount of light straw-colored fluid. Such an exudate is most abundant eighteen hours after the injection of formalin, and may then be 3 or 4 cm. in thickness. Absorption of the exudate usually occurs in a few days, but may be markedly delayed when the formalin is not diluted. A marked proliferation of the connective tissue is concomitant with the absorption of the exudate.

The epithelial covering shows no changes. The exudate is stained red, and mixed with cellular debris, isolated connective tissue cells and leucocytes. All forms of the latter are present, but polynuclear cells, especially the eosinophiles, predominate in the specimens obtained soon after the injection. The number of connective tissue cells (formative cells) varies with the length of time that has elapsed since the injection, and with the strength of the formalin. Active proliferation of the existing connective tissue can be found on the second or third day after injection. When strong solutions of formalin have been used, proliferation is at first delayed, but once started, becomes excessive. A delicately fibrillar fibrin, differing in amount in the various experiments, is found scattered through the exudate. The blood-vessels are enormously congested.

VI. THE EFFECT OF FORMALIN UPON THE MUSCLES.

After the injection of formalin varying in strength from 0.1% to 100% into the leg muscles of dogs and rabbits, it is usually found at autopsy that the chemical has acted upon a circumscribed area of tissue. When strong solutions have been employed, the affected

muscle is brown in color, hard and dry, cutting much like a piece of dried bread. Exudation into the fascia usually takes place and soft boggy lines run through the dry areas. When dilute solutions are used the exudation is more severe, and the area of muscular tissue acted upon by the chemical is dark red and soft and from it drips a slightly bloody fluid. In one dog, I found within the muscular tissue fifteen days after the injection of 4 cc. of 10% formalin, a cyst containing straw-colored serum and a mass of clotted blood. The walls of the cyst had been formed by active proliferation of the connective tissue of the fascia.

Histologically, we have to deal with a myositis of varying severity. Between the muscular fibers dense aggregations of leucocytes are found. In specimens examined twenty-four hours after the injection of the formalin, infiltration is mainly of the polynuclear variety; in older specimens the mononuclears become very numerous. A homogeneous red-stained exudate often mixed with fibrin is found in the intermuscular septa. When a few days have elapsed since the injection of formalin, evidences of connective tissue proliferation manifest themselves. In the course of two or three weeks this becomes excessive. The muscular fibers are sometimes swollen, sometimes shrunken and wavy; they are hyaline and stain bright red. Total loss of nuclei and muscular striation is common. In many places segmentation of the fibers has occurred. Often, especially when strong solutions have been employed, the muscular fibers are shrunken and stain blue with haematoxylin; at other times the musculature stains a uniform deep purple. In these localities no leucocytes can be found. Any or all of the above conditions may be present in a single field of the microscope.

It is not necessary that the formalin be injected into the tissues in order that all the above changes may occur. Changes identical with those described occur in the musculature of the eyelids when formalin is dropped into the eyes, in the abdominal wall after intraperitoneal injections, or in the skeletal muscles after the chemical has been injected subcutaneously.

When formalin comes in contact with non-striated muscular tissue, changes similar to those observed in voluntary muscles result. In the intestinal wall, for example, after the injection of formalin into the peritoneum or into the stomach, the muscle cells are contracted, stain red with eosin or, if the destructive action has been more marked, purple with haematoxylin. A loss of nuclei is very common, and slight leucocytic infiltration is occasionally present.

VII. THE EFFECT OF FORMALIN AND FORMALDEHYDE UPON THE EYE.

Lacrymation and congestion of the conjunctival vessels are present in the eyes of all animals exposed to the vapors of formaldehyde. The intensity of these symptoms varies only slightly with the length of exposure, and seems to depend more upon the concentration of the gas to which the animals are exposed. The symptoms usually subside within twenty-four hours. Histological examination a few hours after exposure to the gas shows only vascular congestion.

The slight cloudiness and pupillary contraction which follow very soon after the injection of formalin, up to the strength of 1-500, into the anterior chamber of the eye, always pass off in a few hours. When a drop or two of a 5% solution are injected definite changes can be found after twenty-four hours. The anterior chamber becomes filled with delicately fibrillar, densely matted fibrin, with which polymorphonuclear leucocytes are mixed. These are most numerous in the angle between the iris and the cornea. The lining cells of the anterior chamber are swollen and often desquamated.

In three animals in which a few drops of 10% formalin were dropped into the eyes no changes except vascular congestion of the eyeball and eyelids resulted. For several hours after the introduction of the formalin there was intense lacrymation.

A single drop of undiluted formalin is sufficient to injure an eye permanently. The changes which result are generally as follows: Immediately after the introduction of the formalin there is lacrymation, blepharospasm and contraction of the pupil. After twenty-four hours the pupil is contracted to almost pin-hole size, and refuses

to dilate after the use of atropine. Intense oedema of the eyelids develops and prevents their closure; the cornea becomes dry and opaque. At the end of three days the lids become less oedematous and their margins may become agglutinated through fibrinous exudate.

The conjunctiva and cornea become infiltrated with leucocytes. In the angle between the visceral and parietal conjunctiva large collections of leucocytes are found; twelve or eighteen hours after formalin has been dropped into the eye the cornea is oedematous and its corpuscles show evidences of proliferation. About the canal of Schlemm there is intense infiltration with polynuclear leucocytes. The anterior chamber of the eye is filled with exudate stained homogeneously red with eosin and mixed with delicately fibrillar fibrin, and large numbers of leucocytes. The intensity of the process is subject to great variation.

As in the eye, the changes in the eyelids do not always give a definite reaction to a definite amount of injected formalin. When the reaction is but slight, there may be only well-marked leucocytic infiltration of the mucous lining of the eyelids; the muscular tissue may assume a brownish color after staining with haematoxylin and eosin; sometimes myositis is present. In very severe cases the mucous membrane is covered by a layer of fibrin, the blood-vessels are markedly congested, the tissues are oedematous, and between the necrotic cells are found masses of fibrin.

In addition to the local effects following the injection of formalin, pathological changes occur in the parenchymatous organs in consequence of the absorption of the chemical from the point of injection. These lesions will now be considered.

VIII. CHANGES IN THE LIVER.

Among the general changes produced by formalin in whatever way it is introduced into the body are those occurring in the liver. These consist essentially of cloudy swelling, varying in intensity associated with vacuolation of protoplasm and destruction of the nuclei; ultimately total destruction of cells may occur.

Changes in the liver may be noted after injections into the peritoneum of even dilute solutions. In guinea pigs after the injection of from 4 to 8 cc. of a 1-1000 solution, these changes are of a mild form; the liver cells are swollen, their outlines obscured and the protoplasm granular; the nuclei show no changes. After the injection of 1% solutions the changes are more severe. The columnar arrangement of the cells is partially lost and definite boundaries between the rows of liver cells cannot be distinguished. The cells are more swollen, the granulation is coarser than normal, and the cell outlines are entirely obscured; the nuclei in a few cases are somewhat crenated. The cells about the central veins stain more lightly than those at the periphery of the lobules. Accompanying these changes is a general vascular congestion; occasionally a leucocyte is found in the tissue.

Intraperitoneal injection of undiluted formalin into dogs results in the production of a diffuse cloudy swelling of the liver; the cells are swollen, the outlines lost and the protoplasm is granular. The nuclei are crenated and of bizarre shapes. Between the rows of liver cells are found a few polynuclear and mononuclear leucocytes. The interstitial tissue contains many nuclei and the blood-vessels are congested. These changes are noted from the second to the fourth day after the injection of formalin.

Subcutaneous injections of formalin are capable of producing only slight cloudy swelling of the hepatic cells. Severe changes cannot be found even after the use of strong solutions.

The most severe changes in the liver were noted in those experiments in which formalin was injected into the lungs or into the stomachs of animals, and after the inhalation of the vapors of formaldehyde. The changes under these conditions are very uniform; the columnar arrangement of the liver cells is almost entirely lost and the cells are enormously swollen, some of them having attained fully three or four times the size of the normal cell. The cellular outlines can rarely be distinguished and the protoplasm is greatly vacuolated and granular. The appearance of fatty degenera-

tion is produced, but the irregular contour of the vacuoles speaks against this view. The nuclei are somewhat swollen but have preserved their smooth contour in many cases; at other times the nuclei are crenated or bizarre in shape. Between the rows of liver cells are many polynuclear and occasionally mononuclear leucocytes. Figures simulating karyokinesis are found, but the exact nature of these I could not determine. It is possible that they represent broken down nuclei or polymorphonuclear leucocytes. An increase in the number of nuclei in the interstitial connective tissue is due either to cell proliferation or to infiltration with mononuclear leucocytes. The blood-vessels are filled with blood. The greatest leucocytic infiltration occurred in a dog into the lungs of which 10% formalin was injected.

A marked contraction of the hepatic cells associated with vacuolation, instead of the ordinarily observed swelling, is found in some of the specimens. In no case was I able to find an increased number of leucocytes in the liver sooner than forty hours after the injection of the formalin. In four of the animals which had inhaled formaldehyde I found areas of focal necrosis in the liver. Such foci are sometimes small and six or twelve liver cells have undergone destruction (Fig. 4); at other times areas as large as half a liver lobule are destroyed. Mixed with the granular debris and the shrunken and broken down remains of liver cells are polynuclear and mononuclear leucocytes. Eosinophiles are rarely found. That regeneration of the cells occurs is indicated by the fact that no histological changes can be discovered in the livers of animals which survive for two or three weeks the intraperitoneal injection of dilute formalin.

Hensen (13) has produced parenchymatous changes in the liver by injecting 1.5 cc. of from 0.5 to 4% formalin into the gall-bladders of cats. The changes varied from slight cloudy swelling to total necrosis of the liver cells. In all cases only a slight inflammatory reaction occurred, and evidences of regeneration were plentiful. My own observations agree well with those of Hensen, and it is of interest

to note that formalin, no matter in what way introduced into the body can produce lesions in the liver similar to those resulting from the injection of the chemical directly into the organ.

IX. CHANGES IN THE KIDNEYS.

Changes in the kidneys are constantly present after the injection of formalin. Definite changes are noted in the kidneys within six or eight hours after the injection of from 3 to 6 cc. of 1-1000 formalin into the peritoneal cavities of guinea pigs. Macroscopically the kidneys are firm, swollen, dark red and bloody. Microscopically, the glomeruli show an indistinct structure. The sepearate cells cannot be distinguished from each other; the nuclei are polymorphous or crenated, and often show figures suggesting karyokinesis. The uriniferous tubules show a variety of changes; for the most part the cells lining the convoluted tubules are swollen, granular, and stained red with eosin, while often a faintly reticular structure can be made out. The cells are swollen to the extent of occluding completely the lumina of the tubules, and the cellular outlines are obscured. The nuclei of the cells are pale and swollen, and some have entirely disappeared. In some areas in the kidney the epithelial lining of the tubules has been lost so that nothing but empty holes remain; sometimes these holes contain a small amount of granular material, at other times, desquamated cells with fragmented nuclei. Often the entire epithelial lining is contracted and found lying free in its connective tissue casing. Some of the tubules are filled by a homogeneous, bluish staining mucus-like material. The changes in the straight tubules are similar to those in the convoluted tubules, the cells being swollen and granular. Often the arrangement of the cells upon the basement membrane is disturbed and sometimes total cellular necrosis has occurred, only the empty connective tissue tubules remaining.

In two guinea pigs, one dying eight, the other twelve hours after the intraperitoneal injection of 4 cc. of a 1% solution of formalin, all of the changes described above are found. No definite

structure can be made out in the glomeruli and intense vacuolation of the cells has occurred. In the glomeruli and in the tubules an occasional polymorphonuclear or mononuclear leucocyte can be seen and blue hyaline material is found in some of the tubules.

The administration of formalin by the stomach or its injection into the lungs produces the same changes in the tubules of the kidney as those just described. The same result follows intraperitoneal injections of undiluted formalin into dogs. The glomerular changes, however, are of a severe type. In one case after the injection of 3.5 cc. of undiluted formalin some of the glomeruli are entirely lost and in their places a small amount of poorly stained granular material is found. In another dog there are found only empty tubules containing red vacuolated bodies, probably the remains of necrosed cells.

In the animals exposed to the fumes of formaldehyde the cells of the uriniferous tubules are the seat of intense vacuolation. Further, the changes already described are found and some of the glomeruli are entirely destroyed. In three of the animals exposed to the fumes of formaldehyde areas of focal necrosis were found in the kidneys. Such foci are usually of small size, and cover an area corresponding to the cross-section of two or three tubules. Mixed with the granular debris and the broken down nuclei of the destroyed epithelium are found mononuclear and polynuclear leucocytes.

X. CHANGES IN THE LUNGS AFTER ABSORPTION OF FORMALIN.

Pneumonia and bronchitis, with or without haemorrhage, are found in the lungs of all animals after the injection of formalin. These changes are of about the same type in all the animals, and do not vary in severity with variations in the strength and the amount of the injected chemical.

The bronchi contain desquamated bronchial and alveolar epithelium, together with polynuclear leucocytes. The lining cells of the bronchi are swollen and granular, and in places partially desquamated. Many of the alveoli contain a finely granular or hyaline

material (oedema) with which are mixed desquamated alveolar epithelial cells and leucocytes. The epithelial cells of the alveoli are swollen and granular, and their nuclei are pale and misshapen. Many of these cells are in process of desquamation. The blood-vessels are congested and contain a large number of leucocytes.

XI. CHANGES IN OTHER ORGANS.

Except for an apparent increase in the number of polynuclear leucocytes, I was unable to find changes in the spleen of any of the animals experimented upon. There was always an abundance of blood pigment present and many of the leucocytes were filled with it. In the heart I found no definite changes. The suprarenals were unchanged.

A distinct eosinophilia was found in the bladders of two dogs. In addition the epithelial cells of the bladder had undergone well-marked necrosis (the cells were swollen and granular, with crenated and broken down nuclei) and desquamation. To what extent these changes were pathological, I am unable to say.

In the spinal cord and brain of four animals examined by Nissl's method, the ganglion cells showed chromatolysis, peripheral and central migration of the Nissl bodies, and eccentric location of the nuclei.

XII. CHRONIC FORMALIN POISONING.

Four animals were subjected to chronic formalin poisoning, receiving a series of intraperitoneal injections of dilute formalin. The results were as follows:

Exp. 1.—Rabbit, wt. 920 grm. Injected 6 cc. of 1-2000 formalin on four successive days. The animal seemed slightly uncomfortable but otherwise well. The rabbit was killed ten days later when no macroscopic changes could be found in the organs.

Exp. 2.—Guinea pig, wt. 765 grm. Injected 4 cc. of 1-2000 formalin on four successive days. The animal lost 27 grm. in weight. Twelve days later the pig had regained its former weight and formalin injec-

tions were recommenced. Injections of 4 cc. of the chemical, beginning with 1-2000 and gradually increasing in concentration, were made every second day. No symptoms except a slight restlessness followed the injections. The animal succumbed after the ninth injection—4 cc. of a 1-625 formalin solution. At death the animal weighed 702 grm. Time of experiment 42 days.

Exp. 3.—Terrier, wt. 25 kilo. The experiment extended over a period of 38 days. The following injections were made, one day at least intervening between the consecutive injections:

Ten injections of 8 cc. of 1-1000 formalin; one injection of 16 cc. of 1-1000 formalin; three injections of 24 cc. of 1-1000 formalin; one injection of 32 cc. of 1-1000 formalin; one injection of 40 cc. and one of 50 cc. of 1-500 formalin. The dog was killed with chloroform.

Exp. 4.—Rabbit, wt. 1675 grm. The animal was observed for 106 days. The following injections were made, one day at least intervening between injections:

Twelve injections of 6 cc. of 1-1000 formalin; two injections of 8 cc. of 1-1000 formalin; one injection of 12 cc. and one injection of 20 cc. of 1-1000 formalin.

I now began to increase slowly the concentration of the chemical, as well as to increase the amount injected. After increasing the strength of the formalin I would at first decrease the amount injected so as to make the transition with as little shock as possible to the animal. In 61 days the amount of the chemical injected was raised from 16 cc. of 1-500 to 40 cc. of 1-150 formalin. After the ninth injection of 1-1000 formalin the animal had lost 75 grm.; thereafter the animal gained in weight and weighed, when killed, 1920 grm. For several days the animal weighed 2026 grm. After a series of injections the rabbit would lose weight; the weight would be regained when the injections were stopped. It was also noticed that an increase in the amount of formalin injected was followed by a corresponding increase in the loss of weight.

On post-mortem examination no macroscopic changes of a type much severer than those ordinarily observed could be found. The kidneys and liver of the dog in Exp. 3 were yellowish. In the rabbit of the last experiment the intestines had a distinctly granular feel, and the mesentery was firmer and thicker than normal. The capsules of the kidneys were also thickened.

Histological examination of the viscera gave the following result: In the first three experiments there is fibrino-haemorrhagic peritonitis such as I have already described. In the third experiment organization of the exudate is well advanced, while in the last experiment there is enormous proliferation of the connective tissue. The mesentery is made up almost entirely of embryonal connective tissue, and the serosa of the intestines and abdominal wall is very markedly thickened. Enormous proliferation of connective tissue has also occurred in the omentum. In places areas of fibrin mixed with red blood corpuscles are undergoing organization. A most striking infiltration with eosinophile leucocytes is present in the mesentery, omentum, serosa and submucosa of the intestines, and in the abdominal wall. Other polynuclear and mononuclear leucocytes are present but they are comparatively few in number. About the smaller blood-vessels and irregularly scattered through the young connective tissue are small, densely-crowded aggregations of lymphocytes.

The capsules of the kidneys, spleen and pancreas of the rabbit in the last experiment are thickened, show active proliferation of connective tissue, and are infiltrated with eosinophile and other forms of leucocytes. These changes, it seems to me, are due to the formalin which came in contact with these organs. It is probably the result of this proliferation of connective tissue and thickening of the serosa that the animals are able to bear without apparent suffering the injection of large quantities of the stronger solutions employed in the later experiments.

Severe cloudy swelling is present in the livers of the animals used in the last three experiments. In the dog there was marked fatty degeneration. Areas of focal necrosis are found in the liver of the guinea pig. In the guinea pig, and in the rabbit, more markedly in the latter, the interlobular connective tissue is infiltrated with small mononuclear leucocytes. Slight leucocytosis is present in the livers of all the animals except the first.

The kidneys are the seat of diffuse cloudy swelling. Fatty degeneration is marked in the kidneys of the dog (Exp. 3). The connec-

tive tissue in the kidneys of the last three animals is infiltrated with small mononuclear leucocytes, but other forms are rarely found. Areas of focal necrosis are found in the kidneys of the guinea pig. In the dog extensive areas in which kidney substance has disappeared are infiltrated with leucocytes of the mononuclear variety (Fig. 5). Many plasma cells are present.

The lungs of the four animals show the slight bronchitis and pneumonia usually present after the administration of formalin. Their spleens contain a large amount of blood and yellow pigment and in the last experiment a large number of eosinophiles.

I take pleasure in expressing my thanks to Dr. Hektoen for suggesting the subject of this paper to me; and for continued interest and guidance during its preparation.

SUMMARY.

The results of this investigation may be summarized as follows:

1. The inhalation of formaldehyde gas in even small quantities is followed by bronchitis and pneumonia. Pneumonia is due to the inhalation of the gas and not to secondary infection.

2. Formalin belongs to that rare group of poisons which are capable of producing death suddenly when swallowed.

3. The introduction of formalin into the stomach is followed by the production of a gastritis which varies greatly in character. The duodenum and upper jejunum may also be involved in the inflammatory process.

4. Intraperitoneal injections of formalin cause peritonitis of a fibrino-haemorrhagic character. A definite reaction is obtained when very dilute formalin (1-1000) is employed. In the peritoneal cavity formalin exercises a destructive action upon all organs (pancreas, liver, peritoneal fat, Fallopian tubes, etc.) with which it comes in contact and causes inflammation in these organs.

5. The lethal dose of formalin when injected intraperitoneally into guinea pigs is approximately 2 cc. of 1-1000 formalin for each 100 grm. of body weight.

6. The injection of formalin into the lungs is followed by pneumonia and bronchitis.

7. The inflammation which follows subcutaneous injections of formalin is characterized by intense exudation.

8. The injection of formalin into the muscles produces myositis.

9. The injection of formalin into the anterior chamber of the eye causes the accumulation of an exudate containing leucocytes and fibrin. When formalin is dropped into the conjunctival sac iritis follows and may be severe enough to destroy the eye.

10. Formalin in whatever way introduced into the body is absorbed, and is then capable of producing lesions in the parenchymatous organs.

11. Changes in the liver after absorption of formalin consist of mild or severe grade of cloudy swelling accompanied by vacuolation of the pretoplasm, changes in the nuclei and leucocytic infiltration. Focal necrosis may result. Similar changes follow the inhalation of formaldehyde.

12. The injection of formalin or the inhalation of the vapors of formaldehyde produces cloudy swelling of the parenchyma of the kidney. Focal necrosis may result.

13. Pneumonia and bronchitis are found in all animals after the injection of formalin.

14. The leucocytic infiltration which follows the introduction of formalin into an organ has these general characteristics: The eosinophiles are the first leucocytes to appear; these are followed by the other polynuclear leucocytes; last appear the large and small mononuclear leucocytes. Similar phenomena occur in the trachea, bronchi and lungs of animals subjected to formaldehyde inhalations.

15. Formalin is, directly or indirectly, chemiotactic for leucocytes. The tissues which are not infiltrated with leucocytes after the injection of formalin are those which have been so injured by the chemical that an inflammatory reaction is impossible.

16. Animals subjected to chronic poisoning with formalin administered by intraperitoneal injection develop fibrinous peritonitis,

associated with marked eosinophilia. The changes in the kidneys and liver consist of cloudy swelling, fatty degeneration, focal necrosis and leucocytic infiltration.

Bibliography.

1. Aronson, H.—*Zeitschr. f. Hyg.*, 1897, xxv, 168.
2. Fairbanks, A. W.—*Centralbl. f. Bakt.*, 1898, xxiii, 20, 80, 138.
3. de Schweinitz, E. A.—*Yearbook of the U. S. Department of Agriculture*, 1896.
4. Kobert, R.—*Goerbersdorfer Veröffentlichungen*, 1898, i.
5. Moeller, A.—*Goerbersdorfer Veröffentlichungen*, 1898, i.
6. Doty, A. H.—*New York Med. Jour.*, 1897, lxvi, 517.
7. Klipstein, E.—*Zeitschr. f. klin. Med.*, 1898, xxxiv, 191.
8. Harrington, C.—*American Jour. of the Med. Sci.*, 1898, cxv, 56.
9. Annett, H. E.—*Lancet*, 1899, ii, 1282.
10. Adler, I.—International Contributions to Medical Literature. Dedicated to A. Jacobi, 1900, 309.
11. Borst, M.—*Verhand. d. Deut. path. Gesellsch.*, 1900, 176.
12. Flexner, S.—Contributions to the Science of Medicine. Dedicated to William H. Welch, 1900, 743.
13. Hensen, H.—*Arch. f. exper. Path. u. Phar.*, 1898, xlii.
14. Benedicenti.—*Arch. f. Anat. u. Phys.*, 1897, 219.
15. Heile.—*Virchow's Arch.*, 1900, clx, 148.

EXPLANATION OF PLATES.

Fig. 1. Eosinophilic pneumonia in a guinea pig exposed for one and a half hours to formaldehyde. (Sec. I, Exp. 1.)

Fig. 2. Pneumonia at a later stage, in a rat exposed for three and a half hours to formaldehyde. (Sec. I, Exp. 2.)

Fig. 3. Eosinophilic tracheitis in a guinea pig exposed to formaldehyde inhalation for three and a half hours. (Sec. I, Exp. 2.)

Fig. 4. Focal necrosis in the liver in a guinea pig killed forty-two hours after exposure for five hours to the vapors of formaldehyde. (Sec. I, Exp. 4.)

Fig. 5. Extensive leucocytic infiltration found in the kidney of a dog subjected to chronic poisoning with formalin. (Sec. XII, Exp. 3.)

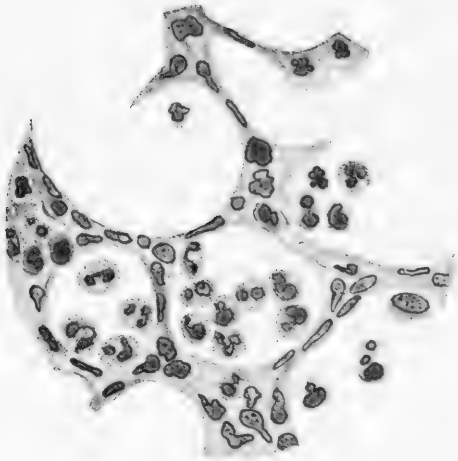

FIG. 1.

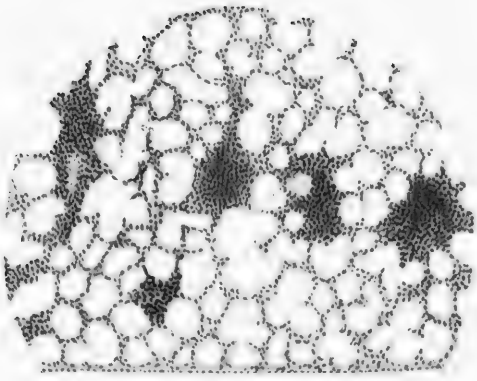

FIG. 2.

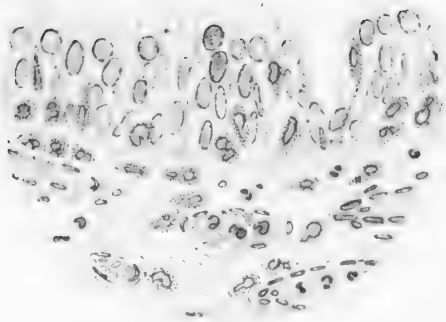

FIG. 3.

FIG. 4.

FIG. 5.

BACILLUS MORTIFERUS (NOV. SPEC.).

By NORMAN MAC LEOD HARRIS, M. B. (Tor.)

(*From the Pathological Laboratory of the Johns Hopkins University and Hospital.*)

It is proposed in this paper to describe a hitherto unknown bacillus which is pathogenic for man and animals.[1]

Source.—From an abscess in the liver of a person who died and came to autopsy in the Johns Hopkins Hospital on October 13, 1900.

Clinical Report.—For the following clinical notes I am much indebted to the kindness of Professor William S. Halsted, under whose care the patient was admitted.

J. B., æt. 44. Admitted to the Johns Hopkins Hospital October 6, 1900. The family and past histories are unimportant.

Present Illness.—Note of October 6: The patient was admitted with a history of headache of four days' duration. Three days before admission nausea began, accompanied with vomiting, which continued until last night. The patient thinks the vomitus had a faecal odor and was dark yellow in color. He was unable to take food until 24 hours after vomiting. Abdominal pain began two days ago and was constant, not paroxysmal, throughout the whole right side. He places his hand to the right of the umbilicus and to the upper right quadrant of the abdomen. The bowels have moved daily three or four times and contain no blood. There was a free movement last night with flatus. He has had chills, one at 8 P. M. last evening and one at 2 A. M. this morning, followed by profuse sweating. The patient never had previous similar chills, and no trouble of the kind is known in his neighborhood. He drinks hydrant water. Temperature 103° F. Micturition normal.

Physical Examination.—Patient walks erect. He is large, well developed, of good color, skin moist, no jaundice, conjunctivae clear. Tongue slightly coated. He complains of intense pain when head is touched. There is no cervical rigidity. Pupils react equally to light. He is rather

[1] Presented at the second annual meeting of the Society of American Bacteriologists, in Baltimore, Md., Dec. 27 and 28, 1900, and at a meeting of the Medical Society of the Johns Hopkins Hospital, on Jan. 7, 1901. A preliminary report was published in the *Bulletin of the Johns Hopkins Hospital*, 1901, xii, 216; likewise in the *Centralb. f. Bakt.*, 1901, xxix, 447.

excitable. Abdomen: symmetrical respiratory movements are present throughout the whole abdomen. Upon deep inspiration he complains of pain in the upper right quadrant. The urinary bladder is not distended. There is no dulness in the flanks. Liver dulness begins at the 6th rib in the mammillary line of the abdomen. Midway between the median line and the umbilicus, dulness proceeds 8 cm. below the costal margin. On palpation the left side of the abdomen is soft and there is no tenderness and no rigidity of the rectus muscle in its lower half. Above the umbilicus, however, the rectus is rigid and pressure causes the patient to wince. The lower part of the right rectus is also relaxed, but there is slight tenderness on deep pressure. In the right iliac fossa, as throughout the whole right abdomen, there is some tenderness with resistance on deep pressure. There is no muscular spasm in this region. As the upper right quadrant is approached, tenderness becomes more marked, rigidity greater, and just above the umbilicus pressure on the rectus causes the patient to cry out. Over the dull area there is definite muscle spasm and great rigidity of the muscles, and this spasm likewise extends to the rectus above the umbilicus and to the other abdominal muscles around the margin of the dull area. No definite mass can be felt beneath the area of muscle spasm. Tenderness extends into the right flank, but the percussion note is resonant. No tenderness exists in either lumbar region.

There are no signs of hernia. The genitalia are negative. Rectal examination shows no tenderness and no mass. Rectal temperature 104° F. Pulse 100 per minute. Respiration 34 per minute. Leucocytes 36,600.

Evening note, 9 P. M.: Patient has complained of pain in right side of the abdomen throughout the afternoon and says that he feels worse than upon admission. Temperature by mouth at 8 P. M., 105.8° F.; by rectum at present, 105.4° F. Pulse between 100-120 per minute. Leucocytes 7000. Patient looks worse. Face is flushed; skin hot; no nausea and no vomiting.

Abdomen.—Respiratory movements present. There is slight distension. Tenderness has increased throughout the right side and the area of muscle spasm has extended further down the rectus and also further downward and outward in the right abdomen. There is, however, no general tenderness or spasm. The area of dulness seems to have decreased (probably due to increased distension).

October 7. Patient's temperature is still high, between 103°-105° F. There is less pain and tenderness in the abdomen. Leucocytes 8000.

October 8. The patient seems more comfortable to-day. Leucocytes at 11 A. M., 8000; at 10 P. M., 16,000. Pulse 98-100. Temperature at 2 A. M., 100.5°; at 10 A. M., 103.5°. Gruber-Widal test, negative.

October 9. Abdomen looks normal. Peliomata striking. There is no special tenderness present. Abdominal walls are held tense, especially over region of the gall-bladder, so that it is difficult to palpate.

Leucocytes at 11 A. M., 20,600.

Leucocytes at 3 P. M. (before operation), 19,000.

Leucocytes at 5 P. M. (immediately after operation), 22,000.

Note by Dr. Mitchell at 3 P. M.: During the last 24 hours there has been a marked change in the patient's mental condition, more or less delirium having developed accompanied with mental dulness. He responds intelligently to questions, but seems much more stupid than on admission. Dr. Halsted saw the patient at 1 P. M. and advised operation. Percussion by Dr. Halsted showed the area of dulness to be exactly as it was upon admission. Otherwise there is little change since yesterday. The abdominal condition has cleared up markedly since admission, muscle spasm having disappeared, leaving only the dulness and a lessened degree of tenderness. The bowels have moved freely and unaccompanied by vomiting. Temperature has been irregular, but high.

Operation by Dr. Mitchell.—Exploratory laparotomy under Schleich's solution plus morphia gr. $\frac{1}{6}$. Demonstration of multiple abscesses in the liver. General anaesthesia. Rupture of abscesses on under surface of liver near the gall-bladder. Packing off with bismuth gauze. Appendix negative. Four abscesses opened and cultures taken. Iodoform gauze drain inserted. Partial closure with silver wire.

Under Schleich's solution an exploratory incision was made through the right rectus muscle. A little clear fluid was seen in the peritoneal cavity and cultures were made. General anaesthesia was induced for exploration, as great pain was caused by touching the liver or parietal peritoneum.

The area of dulness was found to be due to a large right lobe of the liver. The left lobe projected beyond the median line. The liver was large, its edges thin. The area of muscle spasm was indicated by an injected haemorrhagic condition of the parietal peritoneum. On the surface of the liver several abscesses were seen, measuring 1-3 cm. in diameter. One of these on the left lobe was fluctuant, its surface bright yellow. About it was some fibrin as in fibrinous peritonitis. In the right lobe were several other abscesses.

The liver was packed off and the abdominal cavity explored. The

appendix seemed normal. The intestines were pale and distended. There was nothing to suggest primary infection. In exploring the liver ducts, pus was seen escaping from the lower surface of the liver near the cystic duct, and was found to flow from an abscess on the under surface of the liver which had probably ruptured during manipulation. There were about two ounces of thin, foul, bright-yellow pus which was followed by yellow necrotic-looking material. The abscess cavity was packed with a piece of iodoform gauze to protect the intestines. The gall-bladder was not distended and contained no stones. Its ducts were not explored further but were probably patent, as the gall-bladder seemed to empty itself during manipulation.

Bismuth gauze packing was now placed around the lower surface of the liver and peritoneum above. The abscesses in the left lobe were incised and from three drams to one ounce of thin, foul, pale-yellow pus with flocculent necrotic masses evacuated from each. A clamp could be passed into the substance of the liver to a distance of 5-10 cm. The liver seemed as a whole very soft. A strip of iodoform gauze was placed in each abscess. No other abscesses were seen, but the finger could detect uneven places on the surface of the liver beneath the ribs.

The abdomen was partially closed with mattress sutures of silver wire in the muscle, and with subcutaneous silver wire sutures.

October 10. Patient has been fairly comfortable since operation. Temperature has ranged between 99° and 105°. This afternoon patient had a chill and temperature rose from 103.6° to 105°. Pulse 96 per minute. General condition about the same as before operation. Leucocytes at 11 A. M., 24,000; at 4 P. M., 8600.

October 11. 8.30 A. M., leucocytes 16,800; 5.30 P. M., leucocytes 21,000.

Patient was redressed; the dressings were soaked through with a sanguino-purulent discharge. Temperature has ranged from 102-104.6°. He has been drowsy, at times very restless and anxious to go home. Conjunctivae still yellow, and the skin over the whole body has a slight yellow tinge. Patient has constant thirst. He talks rationally at times, but more often is decidedly irrational.

October 12 and 13. Patient has grown weaker and this morning (October 13) can hardly be aroused. Temperature 102.8°. Pulse 120 and very weak. Respirations 52 per minute. Extremities are cold and cyanosed. Dyspnoea is quite marked. General condition much worse. The jaundice has increased. Tremor still very marked. Death at 3.30 P. M.

Autopsy No. 1608.—Pathological Report.—The autopsy was performed by Dr. Eugene Opie on October 13, 1900, at 8.30 P. M., the body having been dead five hours. An abstract only is given here.

Anatomical Diagnosis.—Multiple abscesses of liver, lungs and spleen. Jaundice. Localized fibrinous pleurisy. Enlargement and hyperaemia of portal and retroperitoneal lymph-glands. Laparotomy wound below right costal margin, packed with gauze and extending to the surface of the liver. Acute interstitial nephritis.

The body is that of a large, muscular man, 172 cm. in length. Rigor mortis is present. The skin of the entire body has a fairly deep yellow hue. Below the right costal margin is a linear incision longitudinally disposed into which pass a large number of gauze strips, separated from the edges of the wound by rubber protective. Subcutaneous fat is abundant.

Peritoneal Cavity.—It contains no excess of fluid. The general peritoneal surface is smooth. The gauze drains referred to pass, a few over the upper surface of the liver a short distance, the majority to the lower surface between the liver and the gall-bladder and colon with its mesentery, and are firmly adherent to the surfaces with which they are in contact. These tissue surfaces are dull and in places of a black color. Strips of gauze enter three small cavities in the liver.

The large intestine is considerably distended. The appendix lying in the right iliac fossa is free of any adhesions.

Thorax.—The pleural cavities contain no excess of fluid. The lungs are retracted after removal of the sternum. The pericardial cavity is normal.

The Lungs.—Left Lung.—In general the surface is smooth and of a mottled gray and black color. The pleura over the external surface of the lower lobe, near the posterior border, is dull and in places covered with a thin layer of fibrin. About the middle of this surface is a round projecting area about 3 cm. in diameter; its center is of a conspicuous bright-yellow color from which extend outward irregular anastomosing lines of the same color (distended lymphatics). About the yellow center is a wide area of a deep red color. On section, the tissue below the yellow and red surfaces is firm and consolidated. The yellow central portion is quite irregular in shape; at one point it has softened and there is a small cavity containing thin yellow fluid.

Near the basal edge of the lung are two smaller lesions similar in char-

acter to that just described, the larger about 1 cm. across; in neither has the bright yellow portion softened. The remainder of the lung is crepitant, dry on section, and slightly bile-stained. The bronchi contain a moderate amount of tenacious somewhat blood-stained mucus. The bronchial lymph-glands are slightly enlarged, soft and of a red-black color.

Right Lung.—This differs but little from the left. Over the external surface of the lower lobe near the posterior border, the surface is dull and covered in places with a small amount of fibrin. Near the basal edge is a lesion similar to that already described in the left lung. The central bright-yellow area, about 1.5 cm. across, is very irregular in shape and not softened. Irregular lines extend outward from it below the pleura. The lymphatic glands are larger, but resemble those of the left.

Liver.—Weight 4090 grm. The organ is of very large size. In general the surface is smooth, but is studded, particularly over the upper surface of the right lobe, by low boss-like projections whose center is a conspicuous bright-yellow color, about which is a wide irregular zone of very deep-red color. The surface between is of a deep-red tint but not so deep as that about the yellow areas.

The anterior third of the upper surface of the right lobe is in very large part occupied by bright-yellow areas often confluent, between which the surface is of a very deep-red color. On section through the projecting areas, one finds that they represent abscess cavities in the substance of the liver, surrounded by a zone of very deep injection. Though they vary very much in size (see Plate XLII), the usual diameter of the central yellow portion is about 2 cm.; the zone of injection varies greatly and is often continuous between adjacent cavities. A narrow, irregular, somewhat convoluted zone of solid bright opaque-yellow material surrounds a cavity containing thin, slightly turbid fluid. The formation of these abscesses appears to bear a definite relation to the liver lobulation. In the right lobe where abscess formation occupies a large proportion of the substance of the liver, in the hyperaemic tissue abutting upon the abscesses corresponding to the lobules are minute bright-yellow areas of opaque appearance, in the center of which is the section of a small vessel. These minute areas are separated by tissue of red color in which also on careful examination can be seen a vessel cut across or longitudinally. The yellow zone apparently increases in size, and other areas are made up of small rounded areas about 2 or 3 mm. across, composed of somewhat soft yellow tissue and separated by red lines. Finally the central portion of such a collection of small foci softens and the larger abscess cavities with irregular walls are thus apparently formed. In one part an abscess abutted upon and eroded a large branch of the hepatic vein.

In the relatively normal portions of the liver, lobulation is well marked; the tissue is bile-stained and greenish.

Gall-bladder.—The surface is rough, having been in contact with the gauze packing. The wall is somewhat thicker than usual. It contains translucent viscid bile. The gall-ducts are normal.

No abnormality of the hepatic artery or portal vein was discoverable. In the gastro-hepatic omentum are several lymphatic glands about 1 cm. in diameter, soft, succulent, and on section of a dull red color.

Spleen.—Weight 190 grm. Upon the anterior edge an opaque-yellow, slightly raised area, measuring 0.5 cm., occurs. This area on section proved to be an abscess surrounded by a deep-red zone of splenic tissue.

Intestines.—Duodenum and jejunum are normal. The lowermost 100 cm. of the ileum show well-marked injection of the blood-vessels, giving a red color which is most intense in the neighborhood of the ileo-caecal valve. Peyer's patches and solitary follicles are normal. Appendix and colon are normal.

Kidneys.—Combined weight 450 grm. The two organs resemble each other closely. The capsule comes away readily, leaving a smooth yellow-gray surface upon which can be seen minute red areas. The organs are firm in consistence. The cortex is 7 mm. thick, and is pale gray with a yellowish tinge (jaundice). Striae are visible. Malpighian bodies are conspicuous. The mucous membrane of the pelvis is stained yellow, and below, here and there, ecchymoses occur.

The splenic, superior mesenteric and portal veins when cut open show no abnormality.

Microscopical Examination of Hardened Tissues.—Liver.—There is a general dilatation of the capillaries with blood, more especially in and around the central vein, where the liver cells are swollen, hyaline, and without nuclei. There is a general leucocytosis present and occasionally can be seen small clumps of polymorphonuclear leucocytes, some of which show fragmentation or distortion of their nuclei. Large mononuclear cells are to be found here and there, resembling those described by Mallory as being present in the blood in typhoid fever. They evidently are phagocytic, as they contain nuclear protoplasm. In the capillaries certain material is met with at times which stains with haematoxylin and under the immersion lens is found to be composed of bacteria.

The chief lesions present are, however, smaller or larger areas of necrosis. If large, their outlines tend to be lobulated in character, and if small, they have a rounded appearance. These necrotic areas are made

up of three zones, (a) an outer, of varying width, which may occasionally be wanting, made up of polymorphonuclear leucocytes; (b) a middle zone, usually narrow, composed of irregular or round masses of small size, homogeneous and taking the haematoxylin stain, and under very high power these are seen to be made up of bacteria; (c) an inner or central zone consisting of necrotic liver cells and leucocytes whose outlines can only be indistinctly seen, and odd clumps of bacteria. This central zone stains intensely with eosin. Quite often the dead areas of one lobule fuse with those of others leading to the formation of large irregularly-shaped masses of necrotic liver substance.

As regards the anatomical origin of these necroses, it would seem that they arise close to the central vein (rarely within, unless by the lodgement of a bacterial thrombus), and at times in the middle third of a lobule. Now and then areas of necrosis are discovered apart from the presence of bacteria as far as can be seen in the sections.

Nowhere is there any appearance of repair, the process being an acute one.

Lung.—The section contains an abscess similar in character to the larger ones in the liver, namely, wide necrotic areas surrounded by a zone of polymorphonuclear leucocytic infiltration; leucocytes occupy alveoli. The surrounding tissue shows engorgement of blood-vessels and infiltration with red blood corpuscles. A bacterial thrombus is seen at one portion of the periphery of a vein.

Spleen.—The organ contains a similar abscess. The zone of necrosis is very wide and at the periphery there is an abundant blue staining material (bacteria?); the zone of leucocytes is very narrow. The pulp is distended with blood; the small vessels are dilated.

Kidneys.—In many small areas, particularly about the vessels between cortex and pyramid, are areas in which the interstitial tissue is infiltrated with round and plasma cells. The cells of the tubules are large, sharply outlined and granular.

Bacteriological Report of the Autopsy.—At the autopsy, plate cultures were made in plain agar from the blood of the heart, the liver, liver abscesses, spleen, kidney and peritoneal cavity. The plates were placed in the thermostat at 36.5° C., left for 24 hours and examined. All were found to be grossly contaminated by a variety of B. subtilis, due no doubt to imperfect previous sterilization of the plates. Later on, a second series of plates was made in both plain

and hydrocele-fluid agar from the abscesses in the liver, and grown aërobically and in a hydrogen atmosphere at 36.5° C. for 48 hours.

A rabbit was likewise inoculated intravenously with 0.4 cc. of the purulent content of one liver abscess.

The inoculated plates containing both media which were grown aërobically were sterile at the end of 48 hours. The anaërobic plates were not examined until the end of 72 hours when it was found that all the plain agar plates were sterile, while of those containing hydrocele-fluid agar, the first plate alone gave three foci of growth.

Description of the Colonies.—Arising from each of the three small shreds of necrotic tissue could be seen a small zone, about 5 mm. in diameter, of minute white colonies, wholly lying in the depths of the medium.

These zones were found on weak magnification to be made up of more or less oval and round colonies presenting a finely broken-glass appearance. In color they were light brown-yellow, and they were translucent and quite smooth in contour.

Cover-slip Preparations.—Stained preparations showed the colonies to be made up of bacilli, small but variable in length and thickness and having rounded ends. They occur singly, in pairs and in short chains, the latter often resembling streptococci. Treated by Gram's method they become decolorized, and no spores were discovered. From the plates, plain and hydrocele-fluid agar tubes were inoculated and grown in air and in an atmosphere of hydrogen. Colonies developed in the latter tubes kept anaërobically which showed on cover-slips bacilli identical in morphology with those present in the contents of the liver abscess. The bacilli stain readily in carbol-fuchsin and aniline gentian violet.

Bacterioscopic Examination of Contents of Liver and Spleen Abscesses.—Liver.—Considerable numbers of polymorphonuclear and mononuclear leucocytes were present amongst which vast numbers of bacteria were scattered. Apparently one type of bacteria only occurred, which was of a rod form for the most part, although coccus-like forms have been noted. The bacilli varied in length, less so in breadth; some seemed almost the size of typhoid bacilli, whilst the

majority were much more minute. Occasionally one saw forms with slightly swollen ends, which at times stained heavily; more rarely, short filamentous forms were to be seen, and also curved rods. No spores were noticed, although beaded appearances were often seen, resembling B. tuberculosis. Appearances suggestive of branching were noted at rare intervals. The organisms decolorized quite readily by Gram's method of staining and were devoid of spores.

Spleen.—Owing to possible over-heating during fixation, the organisms, which were present in countless numbers, stained poorly and were swollen and slightly refractile. In consequence they were coarser in appearance than those from the liver abscess and more definite and numerous coccus-like forms appeared.

Fresh pus diluted with Dunham's solution was examined as a hanging drop. No independent motility could be observed, but brownian movement was active.

Study of the Bacillus in Pure Culture.—Since it was found that the organism was not able to grow upon any of the ordinary culture media except when human blood or serum were present, placental blood or hydrocele-fluid was added to such media when possible, and the cultural characters of the bacillus studied under those conditions. To avoid the danger of working with mixed cultures from the original plate, the organisms obtained from that source were at once plated both in plain and hydrocele-fluid agar and brought under aërobic and anaërobic conditions for 72 hours at 36.5° C. At the end of that period no growth whatsoever was obtained in the aërobic plates, while multiplications occurred only on the anaërobic hydrocele-fluid agar cultures.

Appearance of Colonies.—To the naked eye all the surface colonies presented the appearances of small colon bacillus colonies, being about 1-2 mm. in diameter. Under the microscope the surface colonies were yellow-brown, coarsely granular, somewhat reticulated, nucleated, rather thick at centers, delicately fringed, translucent and with regular peripheries. The deep colonies were small, oval, round, triangular or irregular in shape, dark-brown in color, granular and translucent. The odor of plates was decidedly and offensively faecal.

Morphology.—The form of the organisms proved to be identical with those obtained from the pus of the abscesses and the original plate culture.

Subcultures were made as before and only those were positive which were made on hydrocele-fluid agar and grown either in a hydrogen atmosphere or according to the method of Buchner. It was noted that growth was equally good and rapid whether incubated in hydrogen or in an atmosphere from which the oxygen had been absorbed by alkaline pyrogallic acid.

Depending upon the density of the agar, variations in appearance of colonies could be obtained. If the agar were thin, it was noted that a deep colony would be surrounded by a swarm of much smaller ones. This effect was less noticeable in surface colonies, but if the agar was denser no such radiating effect took place.

The general cultural characters of the bacillus are set down in Table I.

Further Biological Characters of the Bacillus.—Although a complete study of all the biological characters of the bacillus could not be carried out, certain observations were made which deserve to be mentioned.

Viability.—One plate culture sixteen days old was no longer capable of transplantation, but, as a rule, it was found that most colonies on other plates died out at the end of a week. Tube cultures gave better results under certain conditions. It was found that deep stab cultures lived much longer than streak cultures, that is, when both were left exposed to the air after growing 48 hours in hydrogen. For example, one stab culture was found viable at the end of 42 days, another at 21 days, but of four tubes 16 days old, only one gave positive results on subculture. Streak cultures had usually short lives; of two cultures three days old, one only survived, but another culture four days old still gave subcultures. It would have been interesting had these experiments been made under continuous exposure to hydrogen, as the comparison between deep stab cultures and streak cultures when kept exposed to the air, showed such great difference in length of viability that it seems probable that the oxy-

TABLE I.

Medium.	48 hours at 37° C.	72 hours at 37° C.	6th day (room temp.)
Hydrocele-fluid agar (slant and stab).....	Along line of inoculation are seen a few scattered, elevated, semi-opaque, gray-white, moist, smooth, round, glossy cols., measuring 1-2 mm. in diameter. Stab growth is vigorous, white, granular, and translucent. There are many gas bubbles in the medium and condens. water is cloudy and has a precipitate.	Unaltered.	Unaltered.
Hydrocele-fluid glucose agar (stab).....	Gas formation vigorous. Surface is covered by a heavy condensation-water growth. Stab growth is vigorous, whitish, granular and broken up by bubbles of gas. Some growth is seen in gas bubble clefts.	More gas formation. Growth everywhere more decided.	Most gas is now absorbed, and growth is seen in the gas clefts.
Glycerine agar (plain)...	No growth.	No growth.	No growth.
Potato (streaked with hydrocele-fluid).....	No visible growth. Cover-slip preparations show no increase.	Idem.	Idem.
Bouillon + hydrocele-fluid.....	Uniformly and faintly cloudy. No scum on surface. An abundant semi-viscid, flocculent, whitish ppt., which on shaking vigorously diffuses with great difficulty, rising up string-like, ¾ the way to surface and then breaking up.	Slightly more cloudy. A froth is seen on surface and gas bubbles are seen rising. Odor faecal.	Less cloudy. The ppt. has increased somewhat.

Litmus milk + hydrocele-fluid.....	General reaction slightly acid. The cream-ring has a greenish-blue tint. No coagulation, but an appearance as if medium were beginning to clear up.	Slight increase in acidity. No coagulation; same appearance as if peptonizing.	There has occurred a total solution of casein, and medium is now almost transparent and of a dark red-purple color.
Ox blood serum	No growth.	No growth.	No growth.
Gelatine + hydrocele-fluid (shake culture)..	.....	.....	No liquefaction. Large numbers of small, round, white, opaque colonies. No gas bubbles seen.
Human blood glucose agar.....	Gas formation moderate in amount. Growth occurs all way down stab. Odor disagreeably aromatic.	.....	Has been further gas formation as shown by increased number of now empty gas clefts filled with growth. Color of haemoglobin brown.
Bouillon + blood.....	Faintly and uniformly cloudy. It has a froth on surface and small gas bubbles can be seen rising. There is a white semi-viscid ppt. which diffuses with great difficulty.	....	Froth has gone. Haemoglobin color lost and replaced by a light dirty brown. Medium more cloudy than before.
Litmus milk + blood...	Acid reaction moderate. No coagulation.	.....	Reaction unaltered. No coagulation, no peptonization. Color of haemoglobin has nearly been lost.
Dunham's solution + blood	Very faintly clouded. Has a small amount of white, semi-flocculent, semi-viscid ppt. which diffuses with difficulty.	....	Still faintly cloudy. The haemaglobin color is still a rich cherry-red. The ppt. has increased in amount.

Motility, negative. Gram, negative. Indol, trace. Spore-formation, not observed in any medium.

gen of the air exerted no feeble bactericidal influence upon the bacilli.

The organism, too, finally developed a tendency towards reproductive enfeeblement, as subculture succeeded subculture, so that it became more and more difficult to induce it to grow, and, at last, it died out at the twenty-third generation.

Thermal Death-Point.—The tests were carried out with a vigorous hydrocele-fluid broth culture three days old, by introducing small quantities into small Sternberg bulbs and enclosing the bulb in a fine-mesh wire box, immersing it completely in water at a given temperature and constantly keeping it moving, at the same time carefully keeping the temperature even. After exposure, the contents of the bulb were expelled into tubes of hydrocele-fluid agar and plated, then incubated for four days in hydrogen at 36.5° C. A control from the broth culture was also made subject to the same technique, excepting, of course, any exposure to heat beyond that required to expel the contents from the bulb. The results were as follows:

Exposure.			Result.
5 minutes	at	50° C.	= Positive, 15 colonies.
5 "	"	55°	= Negative.
5 "	"	60°	
5 "	"	65°	
10 "	"	50°	
10 "	"	55°	
Control			= Innumerable colonies.

Hence, a five-minute exposure at 55° C. was regarded as the thermal death-point of the bacillus.

Toxin Production.—An attempt was made to determine whether the organism yielded soluble toxins or not. To this end, 550 cc. of dextrose-free broth, made according to the directions of Theobald Smith, of a reaction of .5 +, were mixed with 185 cc. of hydrocele fluid under aseptic precautions and poured into a Fernbach flask and incubated for 48 hours at 36.5° C. Being found sterile the fluid was now inoculated by emptying into it the whole of a hydrocele-agar

culture of the bacillus obtained from the liver of Rabbit VI. Hydrogen gas was then passed through the flask for three-quarters of an hour and the flask was placed in the thermostat. At the end of 24 hours the growth was tolerably abundant, and upon the fourth day it was very heavy, appearing as a slimy, gray-white layer on the bottom of the flask and clouding moderately and diffusely the supernatant fluid. When thirteen days old the culture was filtered through a Pasteur-Chamberland filter. The filtrate was found upon titrating a portion to have a reaction of 0.85 +, being an increase in acidity of 0.35. A rabbit was given 1 cc. of this filtrate intravenously, and during a period of sixteen days during which it was under my own observation the animal showed no symptoms. Twelve days later the animal was found dead, but owing to the carelessness of an attendant its death was not reported. It would appear from this experiment that no active soluble toxin was produced.

Production of Sulphides.—During the attempt to find a soluble toxin it was thought best to renew the anaërobic condition in the flask by passing fresh hydrogen through the culture. As soon as an opening was made a most foul-smelling outrush of gas occurred, and so powerful was the stench that it could be easily detected throughout the building in which the experiment was being carried on. Suspecting the existence of sulphides, a test was made for their presence in the escaping gas, and a piece of filter paper soaked in a weak aqueous solution of lead acetate was very quickly turned a deep brown color, thus giving positive evidence of their presence. As a control to this a similar piece of paper was held in the stream of hydrogen from the generator for two minutes without showing the least trace of discoloration. When filtered, the filtrate gave no evidence of sulphides in solution when lead acetate paper was soaked in it. The gas collected in fermentation tubes also gave positive tests for sulphides, the atmosphere of the culture having been nitrogen.

Fermentation.—Having previously noted the constant appearance of gas in hydrocele-fluid agar made up with dextrose-free broth, an attempt was made to find out how active this fermentation might be, and what was the composition of the gas. Three fermentation tubes

were filled with hydrocele-bouillon whose reaction was 0.1 +, and inoculated with the organism from an active culture, and incubated four days in a Buchner jar. In each tube 1 cm. of the upper part of the branch was occupied by gas, whose composition when roughly estimated was $\frac{3H}{1CO}$ although with this was mixed a gaseous sulphide, presumably hydrogen sulphide, as shown by lead acetate paper. What was the fermentescible substance present in the bouillon? As the sugar, it was believed, has been quite removed from the meat extract by previous fermentation with B. coli, it seems not unreasonable to consider that the gas was produced from proteid, especially as it was sulphur-containing. An analogous condition is seen in the abundant gas formation in dextrose-free broth by the action of Bacillus aerogenes capsulatus.

It might be mentioned that egg-cultures could not be obtained, although twice attempted.

Optimum titre of media.—Several tests were made and it was found that the degree of acidity recommended by the Bacteriological Committee of the American Public Health Association, namely, 1.5, was too great and a growth was difficult to obtain in a medium of that reaction, while the organism flourished most vigorously in media between 0.5 and 0.1 acid.

Animal Inoculations.—As previously stated, a rabbit was inoculated intravenously with 0.4 cc. of pus from one of the abscesses in the liver of the human cadaver. This animal died in seventeen hours and showed no naked-eye lesions, and cultures from it were negative; yet the histological examination of the liver showed interesting changes which will be mentioned later on.

With pure cultures of the bacillus further inoculations were carried out upon rabbits, guinea-pigs and mice with results which practically reproduced in many of the animals the hepatic lesions found in the human case, the organism seeming to exercise a selective action upon the liver when introduced into the blood directly or through the abdominal cavity. The results of this series of inoculations are given in the following table:

TABLE II.

Animal.	Date of inoculation.	Material used.	Quantity of same.	How inoculated.	Results.	Cultures (Pos. or neg.)	Length of time taken to kill.
Rabbit I.	Oct. 23rd.	Pus from liver abscess of cadaver.	0.4 cc.	Intravenonsly.	Died, Oct. 24th.	—	17 hours.
Rabbit II.	Oct. 25th.	Condens.-water of growth from a 3 day-old agar slant, from col. "B." (Gen. 3rd).	0.5 cc.	Intravenously.	Died, Oct. 31st.	+	6 days.
Rabbit III.	Oct. 25th.	Ditto, from col. "A"—3 days old. (Gen. 3rd).	0.3 cc.	Into liver through thoracic wall.	Died, Nov. 6th.	+	12 days.
Rabbit IV.	Nov. 4th.	Condens.-water growth on agar 4 days old, from perit. cav. Rabbit II.	0.25 cc.	Intravenously.	Died, Nov. 10th.	+	6 days.
Rabbit V.	Nov. 4th.	(Ditto).	1 cc.	Subcutaneously.	Died, Dec. 3rd.	—	29 days.
Rabbit VI.	Nov. 11th.	Perit. fluid from Rabbit IV.	1 cc.	Intravenously.	Died, Nov. 16th.	+	5 days.
Rabbit VII.	Nov. 27th.	Condens.-water growth on agar 13 days old, from Rabbit II.	1 cc.	Intravenously.	Died, Dec. 5th.	—	8 days.
Rabbit VIII.	Dec. 5th.	Toxin, 13 days old.	1 cc.	Intravenously.	Died, Jan. 2nd, 1901.	..	28 days.
Rabbit IX.	Dec. 13th.	Bouillon cult. 3 days old.	0.75 cc.	Intravenously.	Died.	—	20 days (?)
Guinea-pig I.	Oct. 25th.	Condens.-water growth on agar 3 days old, from col. "C." (Gen. 3rd.)	0.5 cc.	Intraperitoneally.	Died, Dec. 13th.	—	49 days.
Guinea-pig II.	Nov. 4th.	Condens.-water growth on agar 4 days old, from perit. cav. Rabbit II.	0.5 cc.	Intraperitoneally.	Died, Nov. 8th.	+	4 days.
Guinea-pig III.	Nov. 18th.	Ditto, 2 days old, from liver of Rabbit VI.	0.5 cc.	Intraperitoneally.	Died, Nov. 20th.	+	2 days.
Mouse I.	Oct. 25th.	Ditto, 3 days old, col. "B." (Gen. 3rd).	0.3 cc.	Subcutaneously.	Died, Nov. 6th.	+	11 days.

The gross pathological appearances following upon the inoculation of the animals were so uniform that it seems preferable to give a composite description of the results, rather than to enter into the details found in each animal. Due mention will, of course, be made of any peculiar conditions existing.

After intravenous and intraperitoneal inoculations in rabbits there was rapid emaciation. The chief pathological effects, however, were noted in the peritoneum, liver and spleen.

The peritoneal cavity showed in some cases an acute fibrino-purulent or sero-fibrino-purulent inflammation. The small and great intestines were glued together and attached to the liver, stomach and spleen by fibrinous adhesions, while at times a collection of blood-stained turbid fluid, in which floated small masses of coagulated lymph, appeared in the dependent parts. The vessels of the peritoneum everywhere were deeply injected, but the glossy character of the tissue was rarely lost.

The surfaces of the liver were almost regularly covered by a layer of yellowish-white fibrin which caused the organ to adhere closely to the diaphragm, and the various lobes to one another. On removing this exudate the liver substance was seen to be irregularly studded with yellowish-white, roundish areas which stood out prominently in the dark chocolate coloring of the relatively normal tissue. These areas varied in size from 0.25-0.3 mm. and at times protruded above the general level. On section of the organ it was found that the contents of these foci consisted of a cheesy material. There was no definite zone of inflammation surrounding them, but at times a delicate grayish capsule more resistant to invasion could be seen limiting the larger abscesses. The relatively unaltered liver tissue was softer and much more friable than normal and the lobulations could be made out with difficulty. The gall-bladder was unaffected and usually contained a dark green-colored bile.

Abscesses were absent in the spleen and the organ was enlarged, brown-red in color, soft and friable, and more or less sheathed in exudate which caused it to adhere to the intestines or costal wall.

One rare lesion was that of thrombosis of a splenic vessel causing infarction of the whole spleen, which then was pale red-brown or salmon color, enlarged, firm, and on section, of a homogeneous and dry appearance.

In two instances the adrenal glands were enlarged and pale and oedematous looking. On section, they showed an almost homogeneous structure and were quite moist; no pigmentary layer could be discerned.

In Rabbit No. III the duodenum contained an abscess within its walls about 5 mm. in diameter. Rabbit No. IV showed many small opaque white areas in the walls of the appendix and in the small gut.

In one case (Rabbit No. III) the stomach contained within its walls towards the pyloric end an abscess measuring 0.5 cm. in diameter.

The pleural and pericardial cavities usually contained a very little clear fluid.

The muscle of the heart was pale. In one case only (Rabbit No. II) was any gross lesion noted, and in this there occurred in the walls of the left ventricle and septum ventriculorum a number of small, opaque-white foci measuring 0.25 to 1 mm. in diameter.

In only one animal (Rabbit No. II) were any foci of necrosis found in the brain. This animal showed in both lobes of brain several round opaque-white areas measuring about 2.5 mm. in diameter.

Subcutaneous inoculation of a rabbit was followed in two days by inflammation of an exudative character. Examination on the fourth day showed a tumor beneath the skin averaging 2.5 cm. in extent. The tumor felt tense and was slightly yielding, and the skin over it was reddened. The animal gradually lost flesh but had a good appetite, and the tumor slowly increased in volume and became firm until upon the 18th day it discharged a considerable part of its contents through the skin having become ulcerated. The exuded material was very thick, its color a yellowish-white and without foul odor. Cultures made from this material were negative, and cover-

slips showed nothing amongst the debris which could be said to be organisms. The abscess continued to discharge and more skin over the upper portions sloughed off, leaving a raw surface 3 x 4 cm. which scabbed over, whilst the lower part of the tumor remained prominent and unaltered. The animal grew more emaciated and weak, the scab became detached, and upon the 30th day the animal was found dead. The autopsy showed the existence of a granulating wound corresponding to the slough mentioned. In the axillary region were two round masses the size of hazel-nuts of a somewhat lobulated character, rubber-like consistence and covered by a thin capsule of fibrous tissue. The contents were yellowish-white and of the consistence of stiff putty. There were no signs of peritonitis; the spleen was not enlarged, but was dark red in color and of soft consistence. The liver showed a few small coccidial nodules, its color was slightly darker than usual and its consistence reduced. Everywhere throughout the organ were seen very many small opaque whitish areas, the largest being about 1 mm. in diameter; cover-slips from these areas showed no bacteria.

Cultures from the liver and spleen yielded B. proteus vulgaris and from the caseous subcutaneous nodule and spleen a diplococcus; none of the typical bacilli were found. These organisms found must probably be regarded as secondary invaders, having possibly entered through the skin lesion.

Guinea-pigs are somewhat less susceptible to inoculation with the bacillus. One of these animals lived 49 days and showed, at autopsy, chronic peritoneal adhesions consisting of firm bands of fibrous tissue which united most of the viscera. A second animal showed necrotic lesions of the lungs. The usual lesions met with in rabbits were also encountered in guinea-pigs.

The effect upon mice was imperfectly studied. One mouse was given a subcutaneous injection of the organism and survived 11 days. The only lesion discovered at autopsy was an abscess at the site of inoculation containing yellow-white cheesy material. Strange to say, this animal, of all inoculated in this study, was the only one in

which the bacillus was recovered from the general circulation. The second mouse inoculated succumbed early to a secondary infection.

Pathological Histology.—The microscopic examination of the experimental lesions showed an almost exact reproduction of the appearances given in the description of the material from the human subject; hence this description will be confined to appearances which were not present in the diseased tissues of the original case and to such which in the animals were more severe than were met with in the human tissue.

Liver.—Rabbit No. I, inoculated intravenously with 0.4 cc. of the pus from an abscess in the liver of the human cadaver; dead in 17 hours. The lesions are early and entirely microscopic. They are confined to the outer or middle zones of many of the lobules and consist of round, densely packed masses of polymorphonuclear leucocytes, among which in rare instances can be found minute masses of bacteria. The liver cells have been utterly destroyed where these foci exist and no trace of them can be found. Contiguous to these or even at some distance from them are irregularly shaped areas of hyaline necrosis of liver cells which take the eosin stain strongly. The nuclei of the hyaline cells may be entirely gone or reduced to bodies faintly staining with haematoxylin, while the endothelial cells of the capillaries show in places karyorrhexis, and may even be obliterated. The hyaline necroses are confined to the outer and middle zones of the lobules. Nothing definite was made out as to their origin. The hyaline areas are free from leucocytic infiltration, excepting that which is general throughout the blood capillaries.

In the case of the subcutaneous inoculation (Rabbit No. V) the lesions are conceivably due to toxin action alone, since no abscesses or bacterial foci could be found anywhere, the disease being limited for a long period to the subcutaneous tissues and axillary glands only. The liver of this animal shows coagulative necrosis in which, in some instances, the cells present a fibroid appearance and in others are shrunken or have disappeared. In the latter conditions there is an accompanying infiltration of phagocytic cells, which themselves

occasionally become necrotic, possibly through toxin activity. Exceptionally, such a focal necrosis is quite replaced by mononuclear leucocytes, in which case the picture recalls that of the so-called lymphoma of the human liver in typhoid fever. Occasionally a definite ingrowth of the connective tissue of Glisson's capsule can be seen between some of the lobules, nipping off and destroying parenchymatous cells.

The most interesting materials for study were derived from Rabbits No. III and No. IV (see Table II). These animals showed the typical abscesses, and in addition an overgrowth of the connective tissue cells of Glisson's capsule which invaded the lobules and ultimately destroyed them, as can be noticed upon tracing the course of events throughout the specimen as a whole. The picture is very like that seen in intralobular cirrhosis in man, except for the fact that the process here is not so general in its distribution, but is confined largely to the neighborhood of the abscesses. The intestinal lesions of Rabbit No. IV were found to be localized in the lymph nodes of the submucosa, which had undergone coagulative necrosis. Bacteria were not found in the intestinal lesions, and it would therefore appear as if the process were due to absorption of toxic products from the acutely inflamed serosa.

The spleen of Rabbit No. II is the seat of anaemic infarction, due to the large and important splenic vessels having become thrombosed. The spleen was also embedded in a thick envelope of newly formed connective tissue, rich in blood-vessels, plasma cells and in small mononuclear lymphocytes. In this connective tissue sheath lies a vein of moderate size containing a well-developed platelet thrombus hanging free in the lumen and completely encircled with a layer of endothelium. To one side of the thrombus, a freshly-formed mass of platelets and leucocytes anchors it to the wall of the vein, doubtless the free-hanging end of the main mass being further down in the vessel.

Rabbit II also presented some features which were not met with either in the human subject or in any of the other experimental

animals, namely, abscess formation in the cardiac muscle and in the cerebral hemispheres. In the heart wall three areas were discovered which showed some slight variation in character. One presented a relatively large central zone of coagulation necrosis with good preservation of the general architecture of the muscle fibers but loss of minute detail of structure. The middle zone consisted of a narrow band which stains deeply in haematoxylin and is made up largely of dense masses of bacteria and disrupted cell nuclei. There are very few leucocytes found in this zone. The outer zone, also narrow, is made up of muscle cells which have undergone hyaline degeneration with loss of their nuclei, and which are under considerable pressure from the growing mass within. Beyond these zones the muscle fibers are relatively widely separated, possibly due to local oedema, while the fixed connective cells are swollen and proliferating in places. The other two foci are probably an earlier stage than the foregoing, and only two zones are present: an outer narrow one of hyaline transformation, and an inner larger one of bacteria, degenerated leucocytes and other cellular elements, but in which no definite muscle structure is to be seen. The small blood and lymph vessels in the neighborhood show distinct evidences of a local leucocytosis, although there is no massing of the cells in close relation to the destructive process. The leucocytes are very sparingly and evenly distributed in the area of local oedema.

The brain lesion is marked by a most extensive coagulation necrosis, in which a few polymorphonuclear leucocytes can be seen in stages of disintegration. Bounding this necrotic focus is a more or less complete zone of dense leucocytic infiltration, consisting chiefly of polynuclear cells, although some mononuclear cells are also present. The lymphatic channels of the pia mater near to the lesion are crowded with white blood-corpuscles, while the blood-vessels of the pia and surrounding cerebral tissue show a greater number of leucocytes than are normally present. The bacteria are found around the periphery of the necrotic zone, for the most part in dense agglomerated masses.

Comparison with Species Already Described.—Aside from the anaërobic bacteria producing tetanus, quarter-evil, botulism, malignant oedema and emphysematous gangrene, with which the organism described in this article clearly has little in common, we shall turn for comparison to the published descriptions of the bacteria concerned in the production of gangrene and fetid abscesses connected with the genito-urinary tract, the intestinal tract, the middle-ear, the cranial cavity, and other portions of the body, to determine whether Bacillus mortiferus is or is not a new species.

Veillon and Zuber have described a considerable number of bacterial species, which were isolated in dextrose agar after the method of Liborius and consisted of bacilli, micrococci and spirilla. A brief description of the various bacillary species will now be given.

Bacillus ramosus (Veillon and Zuber) is a small, slender bacillus which is slightly larger than the bacillus of mouse-septicaemia when found in the smears made from the pus or other exudates; but when examined from cultures the bacillus appears in short chains, forming pairs like the letter V, and in pseudo-filaments, and in forms which show irregular swollen contours, resembling somewhat Bacillus diphtheriae. Moreover, according to Guillemot, the bacillus is often branched. It does not form spores, is non-motile, and it stains irregularly by Gram's method. The optimum temperature of growth is 37° C., and no growth was obtained on gelatin at room temperature. On glucose agar the colonies resemble Streptococcus pyogenes but are finer and more transparent. Sugar-bouillon becomes clouded and exhibits a slight whitish precipitate, with a varying amount of gas production. A fetid odor is given off by all cultures. Growth takes place vigorously in hydrogen and the bacillus lives about one month in cultures. The bacillus is pathogenic for the mouse, rabbit and guinea-pig upon subcutaneous inoculation, following which, well-defined abscesses occur leading to the death of the animals from cachexia in about a week, and after intravenous injection of the rabbit an intoxication only results, no lesions having been found anywhere in the body.

Bacillus fragilis (Veillon and Zuber).—This organism is much smaller than the preceding. It is, as a rule, straight, but occasionally is curved. The ends are rounded, and sometimes, on account of the center taking the stains less deeply than the poles, it resembles a diplococcus. In pus it is often difficult to find because the debris takes the stain along with

the bacillus; it does not stain by Gram's method and no spores are formed, although certain swollen short forms of the organism may be mistaken for them. The organism grows in both agar and gelatin at room temperature and does not liquefy the gelatin. The optimum of growth, however, is that of the thermostat. In sugar broth the growth is relatively abundant and no gas is formed; but in glucose agar a small amount of gas becomes evident, although it is insufficient to break up the jelly. Its odor is fetid. Duration of life in cultures is variable; occasionally it lives for 25 days. The bacillus is pathogenic when inoculated subcutaneously in guinea-pigs, causing abscesses which produce death in about eight days. It is much more pathogenic for rabbits, causing large abscesses which slough through the skin, the animals dying in seven or eight days. Inoculated in the veins, these animals die from a cachexia and the bacilli are not recoverable in cultures.

Bacillus fusiformis (Veillon and Zuber). As seen in pus this bacillus is a fusiform rod with pointed ends, occurring often in pairs. In cultures it preserves largely these characteristics, and in addition elongated, swollen and granular involution forms appear. The bacillus is nonmotile, stains poorly with the ordinary dyes and not at all by Gram's method. It grows at room and body temperatures. Gelatin is not liquefied; very little foul gas is formed; broth is rapidly and strongly clouded; the colonies on agar resemble those of B. coli only they are more transparent. The viability lasts only four or five days. The bacillus is pathogenic for the rabbit and the guinea-pig, causing upon subcutaneous inoculation small abscesses without producing death.

Bacillus furcosus (Veillon and Zuber).—A very small bacillus of peculiar shape, dividing at one end into two branches like the Greek letter γ. In cultures the rods are longer and the ends of the branches become swollen into round or pyriform shaped bodies. The bacillus is nonmotile, stains well with the usual dyes but not by Gram's method, does not grow at room temperature, and requires three or four days to produce visible growth at 37° C. The colonies on the surface of agar resemble those of the pneumococcus. In broth the bacillus forms a fine precipitate and some gas, which has a sour and fetid odor. The organism lives in cultures about twenty days. It is pathogenic for guinea-pigs, producing abscesses upon subcutaneous inoculation, without as a rule causing death. Occasionally they die after the lapse of several weeks from cachexia.

Bacillus serpens (Veillon and Zuber).—Somewhat larger than Bacillus fragilis; it stains with the ordinary dyes, at times the center staining

less readily than the ends, whereby an appearance of spores is given. In fact, spores are not formed. The bacillus decolorizes by Gram's method of staining. It grows in the various culture media, but not invariably, as pairs, pseudo-filaments, and short chains. It has, according to Guillemot, an undulatory or serpentine motion in gelatin cultures, which it renders fluid. The optimum temperature for growth is 37° C., but it slowly multiplies at room temperature. The colonies on the surface of glucose agar resemble those of the pneumococcus. Sugar broth is rapidly clouded and a heavy whitish precipitate forms at the bottom of the tube, leaving the medium clear. In a liquefied gelatin stab culture the deposit of a white flocculent sediment takes place, leaving the fluid clear. Fetid gas is formed in small amount in gelatin and agar. Viability extends to twenty or twenty-five days. The bacillus is less pathogenic for mice, rabbits and guinea-pigs than Bacillus ramosus.

Bacillus funduliformis (Hallé).—This bacillus was found in the vagina in normal and pathological conditions, and in other localities. It is a small, usually curved rod with rounded ends. Cultures often show pleomorphic forms appearing as large swollen rods, curved tortuous branching filaments or ball-like masses. In exudates the bacillus occurs chiefly within the pus cells as masses or as disseminated organisms. It does not stain readily with the ordinary dyes, and decolorizes by Gram's method of staining. Its optimum growth is at 37° C., at which temperature about six days are required for it to become very noticeable. It forms gas in sugar media which has a very fetid odor. When grown in broth in a vacuum it clouds the medium slightly at the end of the third day, but by the tenth day the bouillon becomes almost clear. The odor is quite foul. Rabbits are insusceptible, while subcutaneous inoculation of guinea-pigs gives rise to abscesses which at times become gangrenous; introduced into the peritoneal cavity of guinea-pigs no action is noticeable, and in no case did any of the animals succumb.

Bacillus nebulosis (Hallé).—A small bacillus resembling the bacillus of mouse-septicaemia. Usually straight, it curves occasionally or appears as a rod swollen at the center and tapering at the extremities. It decolorizes by Gram's method, is asporogenous, and shows no involution forms. Growth at 37° C. is slow and no growth is obtained at room temperature. No gas is formed in sugar media. It is inconstant in its pathogenic properties, as it produces abscesses in rabbits and guinea-pigs occasionally. It caused the death of one rabbit.

Bacillus caducus (Hallé).—Incompletely studied. A small bacillus which stains deeply by Gram's method and exhibits no pleomorphism. It survives in cultures three or four days.

Bacillus radiiformis (Rist and Guillemot).—An organism closely related to Bacillus serpens in all its characters; possibly identical.

Bacillus thetoides (Rist and Guillemot).—Closely related to or identical with Bacillus funduliformis.

Unnamed species.—According to Rist, Veillon and Zuber previously described two bacilli under the heads of species "A" and "C." The former is a long, slender, poorly-staining, Gram-negative bacillus. It does not grow in gelatin at room temperature, but grows in bouillon at 37° C. without clouding, forming small flocculi and giving off a fetid odor. It forms no gas in any of the sugar media. Injected into guinea-pigs and rabbits subcutaneously it forms small abscesses but does not kill.

Species "C" is a non-motile bacillus resembling the bacillus of chicken cholera. It appears not to grow in chains, but in sugar agar long and filamentous forms with round or fusiform swellings are noticeable. Bouillon is not clouded, growth occurring in small flocculent masses which collect at the bottom of the tube. All cultures have a fetid odor suggesting decaying cauliflower. The bacillus is destroyed by moist heat in one hour at 53° C. The pathogenic properties of the bacillus are manifested upon subcutaneous inoculation of mice, guinea-pigs and rabbits by the formation of abscesses which have a fetid smell. Mice and guinea-pigs survive the inoculations, but a rabbit succumbed after severe reaction. The bacilli were recovered from the local lesions and, in the rabbit, from the heart's blood. Rist encountered both these species and thinks that the latter organism may be identical with Bacillus funduliformis of Hallé.

Guillemot partially describes another species which, being as yet incompletely studied, he terms species "A." It is a small rod about the size of Bacillus ramosus but slightly shorter and in cultures is distinguished by forming chains. At the center of the units of a chain one finds a swelling of a round or fusiform shape, which is not of the nature of a spore. The surface colonies on glucose agar are like those of the pneumococcus. Bouillon is not clouded, but forms flocculi which settle at the bottom of the tube.

Cottet, in addition to finding many of the foregoing bacilli, describes three more which he believes to be new. They have not been named by him and are, in consequence, designated by the letters B, C and D. Bacillus "B" is described as being rectilinear, slightly swollen in the middle, tapering at the ends, occurring occasionally in chains of six to twenty units, and rarely in filaments. It is non-motile and decolorizes by Gram's method. It does not grow in gelatin at room temperature.

No mention is made regarding gas production. Cultures survive six weeks. The organism is pathogenic for guinea-pigs, in which upon subcutaneous inoculation it produces abscesses. Bacillus "C" is a regular, slender strepto-bacillus, the chains of which consist of 10 to 15 units. The bacillus is non-motile and stains by Gram's method. A complete study of the bacillus was prevented by its having died early in cultures. Bacillus "D" is a moderately sized, non-motile bacillus with rounded ends, staining poorly with ordinary dyes and decolorizing by Gram's method. It survived in mixed cultures for a long time, but not having been obtained pure, it was not completely investigated.

A comparison of the above series of bacilli with the species described in this paper, as Bacillus mortiferus, leaves no doubt of the fact that the latter organism differs widely from them all. Among the striking differences are the extremely selective character of Bacillus mortiferus, which requires a medium for its growth containing human blood or serum, its more energetic gas production, and its greater and peculiar pathogenic qualities.

Summary.—From a case of hepatic abscess in man a strictly anaërobic bacillus was obtained in pure culture which is not to be identified with any species of pathogenic bacillus hitherto described. This bacillus is closely adapted to the human organism but it is not wholly limited to growth upon or within that organism, since it exhibits a well-marked and strikingly peculiar action upon certain laboratory animals. There would seem to be justification for the erection, in the case of this bacillus, of a new pathogenic species which I propose to call Bacillus mortiferus.

EXPLANATION OF PLATES.

Plate XLII.

Fig. 1. Drawing of a section of the human liver, showing different stages of the necrotic process. Natural size.

Plate XLIII.

Fig. 2. Central necrosis of a lobule in the human liver. The dark portions around the periphery of the necrotic area are bacteria. × 75.

Plate XLIV.

Fig. 3. A portion of the liver of Rabbit No. iii, showing, (a) necrotic material, (b) formation of dense fibrous tissue, (c) lobules more or less involved by invading fibrous tissue arising from Glisson's capsule. × 20.

Fig. 4. Smear preparation of Bacillus Mortiferus.

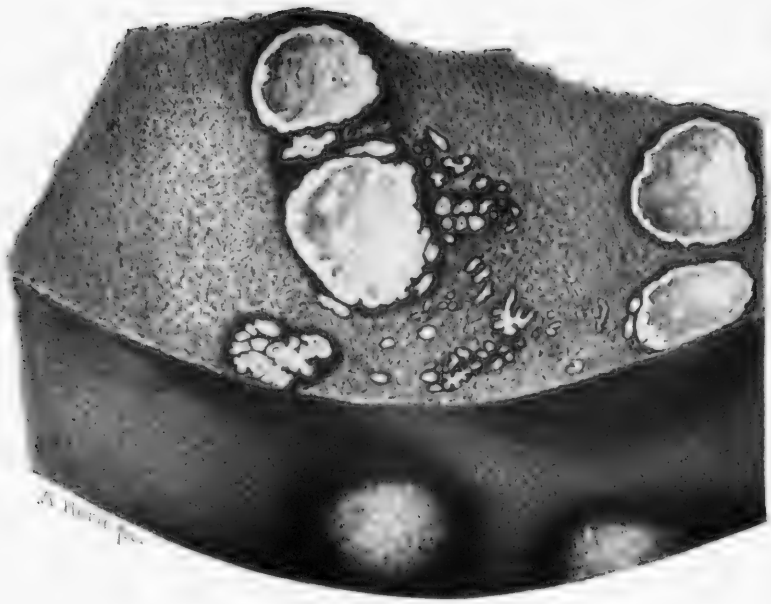

FIG. 1.

FIG. 2.

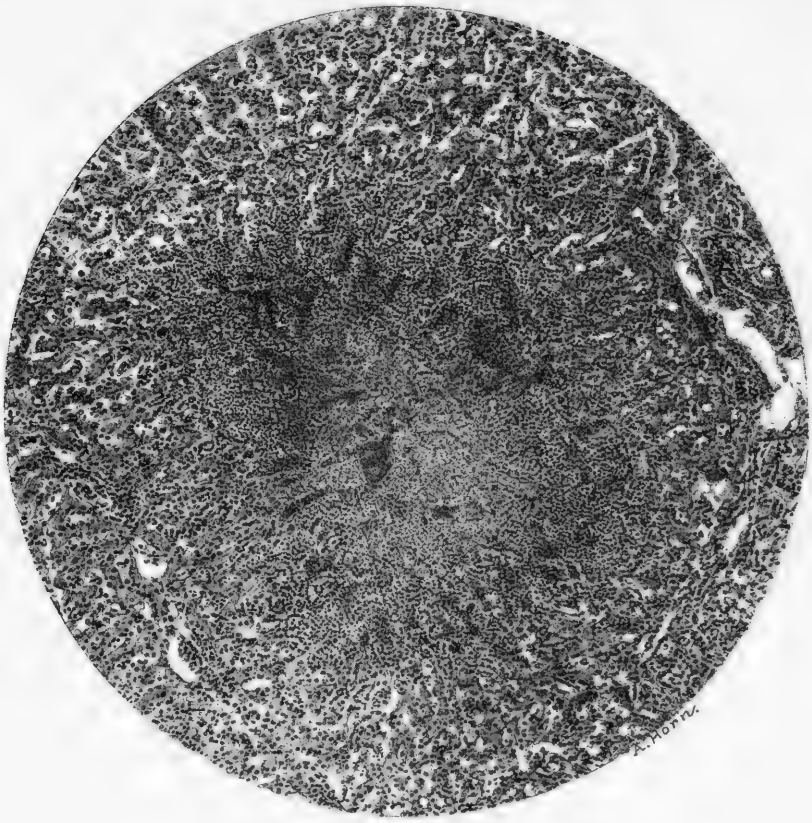

FIG. 3.

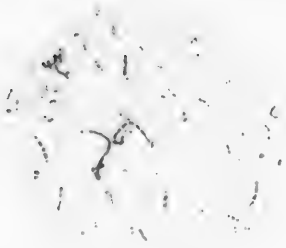

FIG. 4.

BIBLIOGRAPHY.

Veillon.—Sur un microcoque anaérobie trouvé dans les suppurations fétides. *Soc. de biologie,* 1893, 807.

Veillon et Zuber.—Sur quelques microbes strictement anaérobies et leur rôle dans la pathologie humaine. *Soc. de biologie,* 1897, 253. *Arch. de méd. expér.,* 1898, x, 517.

Hallé.—Recheches sur la bactériologie du canal génital de la femme. *Thèse de Paris,* 1898.

Rist.—Etudes bactériologiques sur les infections d'origine otique. *Thèse de Paris,* 1898.

Guillemot.—Recherches sur la gangrène pulmonaire. *Thèse de Paris,* 1899.

Cottet.—Recherches bactériologiques sur les suppurations péri-uréthrals. *Thèse de Paris,* 1899.

NOTE. Since the publication of the preliminary report upon Bacillus mortiferus there have appeared in print two articles upon pathogenic anaërobic bacteria. One by Norris,[1] the other by Albarran and Cottet.[2] The former describes a bacillus obtained from a case of abscess of the liver which bears some resemblance to Bacillus mortiferus, but which differs in morphology, cultural and pathogenic properties. The latter investigated the anaërobic bacteria occurring in abscesses of the genito-urinary tract and added no new species to those already given in this paper.

[1] *Journal of Medical Research,* 1901, I, 97.

[2] *La presse médicale,* 1903, xi, 85.

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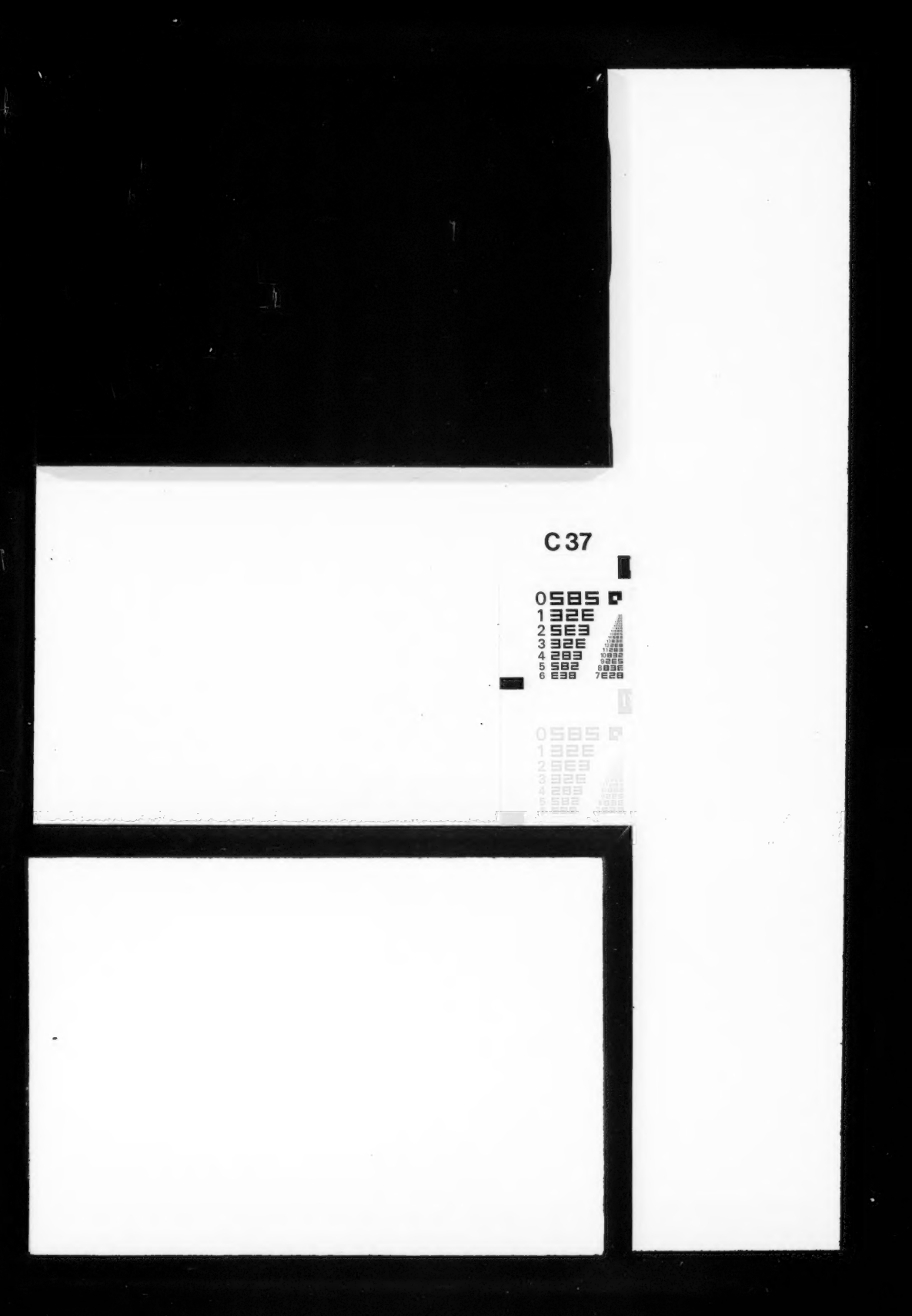
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